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On Being a Medical Geneticist

F. CLARKE FRASER

*Department of Genetics, McGill University,
and Department of Medical Genetics, The Montreal Children's Hospital,
Montreal, Canada*

BEING, I THINK, your youngest President, I cannot hope to use this occasion to give you the benefit of my mature wisdom, and in particular to match the scholarly and elegant presentation of my predecessor (Dunn, 1962). Neither am I willing to play the part of the brash iconoclast I was when I first got into human genetics and (fortunately) was too young for anyone to listen to me. It was suggested that I should call my talk "Ponderings of a Peripatetic Pediatrician," but the fact that I'm not a pediatrician spoils the alliteration. So I will air some thoughts that are either too trivial, or vague, or so completely unsupported by data, that I could not present them anywhere but in a Presidential address.

It's been a great year for genetics, saddened for us by the death of a former President of our Society, Madge Macklin, who played a pioneer role in introducing genetics to the medical fraternity, and whom we all loved for her energy, tenacity of purpose, humour, compassion, and the whimsical tirades with which she would castigate her male colleagues. She did a fine job and had a good time; may her memory remain as an inspiration for us all.

She would have enjoyed the excitement I'm sure we all feel at the dramatic progress that has occurred in genetics in the past year. In particular the "cracking" of the genetic code leaves me speechless with admiration, and I take pride in the fact that human geneticists played an important part in making this possible. Equally fascinating, although not so far advanced, is the analysis of "regulator" and "operator" genes and their control of gene activity. Here, too, human genetics is making contributions in an area which is basic to our understanding of differentiation and development, normal and abnormal. There is also some promise that the genetic code may be subject to controlled alteration, at least at the RNA level — a fascinating and frightening prospect, which, if it ever materializes, will place a tremendous responsibility on the medical geneticist.

Recent progress in human cytogenetics has consisted (necessarily, at this stage) mainly of documentation of relations between karyotype and phenotype, but a neat combination of the cytological and genetic approach has led to the intriguing concept of sex chromosome differentiation as a possible explanation of dosage compensation (Lyon, 1962). A number of groups are combining cytological and genetic observations to map the human chromosomes; the X chromosome map is well under way, and we now have a sex-linked blood group

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(Mann *et al.*, 1962) which has started quite a flurry of linkage studies. Mapping the autosomes is harder, but already there are some slight signs of progress. And there is progress on many other fronts, so rapid that one could spend all his time reading and still not keep up with it.

So Human Genetics is having a heyday. A heyday, incidentally, is "a time of greatest vitality, vigour and bloom, a season of ardor, joy, exuberant spirit and *wildness*." Perhaps a certain amount of wildness is necessary in a period of exuberant growth — it certainly characterized the first such period in Human Genetics, after the rediscovery of the Mendelian laws, with the overenthusiastic attempts to jam the square blocks of pedigree data into the round holes of Mendelian segregation, and the naive hopes of human betterment that Mendelism brought to the Eugenicists.

In this second growth phase we again see examples of those who kick over the traces and go, half-cocked, off the deep end (just as this metaphor has done). Genetics has, indeed, lately acquired a tremendous boost in prestige and an aura of glamour, particularly among our medical colleagues who tend to attribute to it powers that border on the magical. Partly as a result of this, departments of Medical Genetics are springing up in medical schools all over the country (often staffed by personnel without very much training in genetics), enormous amounts of money are being granted for research, and enormous numbers of papers are being published in the name of genetics. As the volume goes up the quality goes down. When does the law of diminishing returns become a limiting factor?

I don't want to detract at all from the impressive accomplishments I have already mentioned. But let us not oversell ourselves, and let us protect the prestige of Human Genetics from jeopardy by unqualified people speaking irresponsibly in its name. It happened before, and it could happen again. Much genetic nonsense is still being written about race (*e.g.*, Putnam, 1961), people are still getting the data to fit Mendelian ratios by such errors as omitting to omit the proband, and still invoking reduced penetrance and other euphemisms for ignorance to account for deviations from Mendelian expectations that can better be accounted for by other mechanisms.

In cytogenetics the applications of the recent advances to clinical problems so far are minimal, being pretty well limited to the identification of high-recurrence-risk trisomies. Identification of sex chromosome aberrations, for instance, although it has increased our understanding a great deal, does not influence the clinical management of the patient in the great majority of cases. Most mongoloids can be diagnosed unequivocally on clinical grounds. Some, at least of the doubtful ones turn out to be mosaics, and it will be helpful to learn how these develop, mentally, in comparison to the usual type. If, on the other hand, cell cultures show normal karyotypes in a suspected mongoloid, how can one be sure it *isn't* a mosaic?

Genetics has contributed a lot to our understanding of human disease, and particularly the inborn errors (although the greatest credit goes to the biochemist rather than the geneticist for these advances), but the specific situation where this knowledge can be directly helpful to the patient or his family includes a fairly small proportion of the cases seen in medical practice. Stating pontifically, at Medical Rounds from time to time, that "this disease has or

chance in four of recurring in subsequent sibs" still seems to impress some of my medical colleagues, but leaves a lot of problems unsolved for the patient. Probably because they are more difficult to analyze, the multifactorial diseases — familial, often common, but not fitting simple Mendelian segregations — have been rather neglected genetically, except for the attempt to fit them into Mendelian pattern by invoking penetrance, and so forth. There are signs of progress on this front, however, and I would like to mention in particular the work of Fraser Roberts and C. O. Carter who are finding evidence in several congenital malformations for a multifactorial pattern of inheritance (Carter, 1961; Roberts, 1962a), and in the case of congenital dislocation of the hip have made a beginning in identifying some of the specific elements in the system.

Let's admit that the DNA-RNA code isn't the whole answer. There are, no doubt, other systems that transmit genetic information (*e.g.*, the cell membrane) that may be very important in developmental processes. As Kacser (1960) pointed out, a computer contains a lot of information besides what's on the tape, and this information differs from computer to computer. It may well be the same with cells, and this would mean that not all familial, intrinsically determined diseases and defects will be traced to alterations in the DNA. The people who claim that a familial anatomical malformation is no different, in principle, than a deformed sickle cell hemoglobin molecule are, I think, oversimplifying the situation, and I doubt that anencephaly, for instance, will ever be identified as a molecular disease.

The present heyday of genetics raises other problems too. How many invitations to speak can you accept without seriously diminishing your working time? Of course we have a responsibility to educate, but there is a limit, or should be. I would suggest that from 50 to 95 per cent of the invitations to speak that we get are from groups who will not noticeably benefit from our donations of time and effort. There are too many medical societies, ladies' auxiliaries, radio commentators, student nurses' classes, physiotherapist associations, etc., who would like you to explain all about this wonderful new subject, genetics, in one hour, and most of us under such circumstances can succeed only in muddling such a group, to our mutual detriment. We could aid the cause of genetics by refusing most such invitations, on the ground that any group really interested in the subject will want to have more than one hour's exposure to it. In case you do have to accept, I would like to pass on a suggestion which can save a lot of time. You only need to have two slides for all such occasions. I use a slightly out-of-focus picture of the wiring diagram on my daughter's radio, and an equally blurred reproduction of a page from the Nova Scotia Tide Tables. This technique can also be used for papers given at scientific meetings when you are not quite sure of your results.

Another source of increasing demands on our time are the requests from medical journals to referee manuscripts with a genetic aspect. This can take a lot of time, but it needs to be done; too many journals do not have a geneticist on their list of referees. We should, whenever possible, take issue with editors who publish genetically false or nonsensical statements, and thus help to improve the reporting of genetic data in medical journals.

A further danger of the genetic heyday arises from the large sums of money

being poured into genetic research these days. I am, naturally, very much in favour of good salaries for geneticists, and adequate funds to support research which often requires funds for expensive equipment, field trips, diagnostic tests, and so forth. But there is a danger of becoming over-equipped. Just as our children are supplied with so many realistic toys, television sets and supervised play periods that they don't seem to use their imaginations any more, we may tend to get so busy using our expensive machines, simply because they are there, programming data so they will fit a computer, filling out application forms to get more money to buy more machines, writing progress reports, and rushing around the country (on expense accounts) seeing how other people are using their machines, that we lose sight of the basic problems, and no longer take time to sit down and think. Yet Crick (1961) made his splendid attack on the code in a room borrowed for a few months from a Zoology museum, and Lejeune (1959) found the mongoloid trisomy in an attic laboratory equipped with one cock for serum, an incubator and an obsolete microscope. He had an idea (one of many) and time to work it out.

One of the most important demands that the heyday is making on us, as geneticists, is to provide training for the rapidly increasing number of people who need it, and this means providing properly trained teachers to do the training.

How, then, should a medical geneticist be trained? Obviously he should have a solid grounding in mathematics, biochemistry, basic genetics, human genetics, and cytology, as well as a medical degree, preferably with a medical specialty such as hematology, neurology or pediatrics. He should be able to relate well to people, have enough psychiatric training to handle the emotional aspects of counseling, and be a good teacher. A working knowledge of anthropology and sociology is desirable. And furthermore, he must live long enough to get all this training and still have a few years left to practice his profession!

The fact is that those who call themselves medical geneticists (or at least work in departments of Medical Genetics) have a wide variety of talents and training, and this is all to the good. Those with degrees in both genetics and medicine can enjoy the prestige of being regarded as geneticists among their medical colleagues, and as physicians among their geneticist confreres, but are kept from becoming unduly conceited by the realization that they are not really expert in either specialty, since each is a full-time job. Some medical geneticists have Ph.D.'s in basic genetics and have gotten into the medical end of it by a variety of routes other than through medicine. Medical schools are rapidly losing their prejudices against non-medical specialists, and lack of a medical degree is no longer a serious handicap to work in Medical Genetics, provided the worker is honest and critical (as he ought to be in any scientific field), and does not oversimplify or underrate the medical aspects of the condition he is studying. Many non-M.D.'s are doing first-class work on the genetics of disease and have the full respect and trust of their medical colleagues. The plant cytogeneticists in particular are having a ball, because of their excellent cytological training, and the fact that they have known for years about many of the things the medical people are astonished to discover in man.

If one extrapolates the present trends it looks as if much of what is now

called genetics may be incorporated into the other disciplines over the next few decades. The teaching of DNA and RNA metabolism and the genetic control of protein synthesis is being taken over more and more by the biochemists. The microbiologists are rapidly moving in on microbial genetics, and it looks as if Departments of Bacteriology may well take over the bulk of teaching and research in this area. The embryologists aren't moving in quite so fast, but eventually they may well take over many areas of developmental genetics. The anthropologists may very well incorporate some aspects of population genetics into their research and teaching. So what's left for genetics, and in particular Medical Genetics?

For one thing there is the special methodology and mathematical background of Human Genetics. So far I haven't seen any signs of anyone taking this over. There is still plenty of need for intelligent, thorough, pedigree-collecting to delineate modes of inheritance more precisely, establish recurrence risks, extend linkage maps, and in general provide the basic material on which so much of our genetic knowledge is based. Much of cytogenetics is likely to remain with us, although hematology and pathology laboratories may well take over much of the diagnostic screening. And, of course, there is genetic counseling.

It is often said that counseling should be done by the patient's personal physician, who, therefore, ought to be adequately trained in at least the basic principles of genetics. This may be somewhat impractical. There are, of course, lots of cases where the pattern of inheritance is clear, and precise predictions of recurrence risk can be established with a minimum of genetic knowledge. But there are complications, such as mutation, reduced penetrance, phenocopies and genetic heterogeneity, that can make even apparently simple cases tricky. Furthermore, there are a great many conditions showing simple Mendelian inheritance that are not generally recognized as such, and the relevant literature is usually not readily available to the average physician. There are no good compendia available that list recurrence risks, and indeed some among us feel that there should not be one, because it may lead the physician who consults it to oversimplify his counseling. (I will argue that it is better for him to oversimplify reliable risk rates than falsify them, as he may do otherwise, but that is a matter of opinion.) In any case the average physician, usually being human, is likely to forget his Mendelian principles after a few years out of school, unless he is using them, and Fraser Roberts (1962b) has estimated that the frequency of cases needing expert genetic counseling would work out at about one case a year for the average general practice. (I suspect this is an underestimate, but not grossly so.) Furthermore, an increasing number of people in this civilization do not have a family physician, and in the usual clinic the doctor has little time to take an adequate family history or to interpret recurrence risks adequately to the patient.

So a medical geneticist, in the most appropriate sense, should be one who is capable of teaching elementary genetics of a kind and at a level suitable for medical students, of supervising the training of graduate students and others who wish to do research in the genetics of human disease, and of acting as a consultant in cases referred for genetic counseling.

In December 1961, the World Health Organization called a meeting of an Expert Committee on Human Genetics to prepare a report on the Teaching of Genetics in the Undergraduate Medical Curriculum and in Postgraduate Training (WHO, 1962). The report can be obtained from Columbia University Press in New York, or the WHO in Geneva. Although the strength of the recommendations of the report was modified somewhat in view of the varying economic and political conditions in different parts of the world, it will be good ammunition for those of us who think genetics doesn't occupy enough space in our medical school curricula. After an introductory section on the value of genetics in the medical sciences, the report discusses undergraduate and postgraduate training, and I would like to summarize what it says about this.

The student entering medical school should already have a thorough grounding in biology including elementary genetics. This is, of course, not always so. In the preclinical years of medicine he should have a short course reviewing basic genetics and introducing the special methodology of Human Genetics. This should take a minimum of 15-20 hours, *providing there has been satisfactory teaching previously*. If not, the course should be lengthened proportionately. The timing would vary with the local conditions. At the University of British Columbia, for instance, the students enter a four year course in Medicine after completing the Bachelor's degree. At the request of several Departments, particularly Biochemistry and Bacteriology, who want the students to have this background for their own teaching, the lectures on basic genetic principles are given in the first year. Principles relating more specifically to Human Genetics are presented early in second year, when the students are becoming more familiar with the concepts of disease and will shortly begin to deal with patients. Incidentally, the Committee almost unanimously agreed that it was didactically good to introduce the course by a description of DNA, RNA and their role in controlling protein synthesis, rather than with Mendelism. This material is not only exciting, but if well grasped provides a logical basis for understanding the concept of the gene, dominance and recessiveness and the disappearing distinction between them, and the role of the gene in disease.

The concepts of expressivity and penetrance seem to be difficult for the medical man to grasp; here it helps to point out that measles, for instance, produces a variable clinical picture in different patients, just as a gene may do, and that the concept of reduced penetrance is very much like the concept of subclinical disease. Population genetics often presents an obstacle to the medical student who for some reason seems to develop a phobia for mathematical abstractions once he enters medical school, but with a little thought the basic concepts can be developed from first principles without writing q^2 on the board even once! Microbial genetics should be included, if not dealt with in microbiology. A laboratory period or practical session is highly desirable, and could include determination of blood groups and color blindness (single gene differences), PTC taste threshold (single gene difference with modifiers giving a bimodal distribution) and a quantitative trait (normal distribution). The sex ratio in families of male and female students, respectively, can be used to illustrate the bias caused by inclusion of the proband. This can be done effectively in class, too, by having a number of male (or female) students state the

total number of boys and girls in their sibships, including themselves, and then omitting the probands to approach the expected 50:50 ratio. There can be microscopic demonstrations of sex chromatin and karyotypes, blood smears of hereditary anemias, and demonstration of simple biochemical phenotypes such as electrophoretic separation of hemoglobins. Patients with hereditary diseases and their pedigrees can be presented, and (as is done at Sao Paulo, for instance) groups of students can each take an inherited condition, work up a case and prepare a short report and literature review. This takes space and a lot of the instructor's time; few of us are able to do this at present, but it is something to aim at.

In the clinical years there are many opportunities we shouldn't miss to demonstrate the application of genetic principles to human disease in the clinics and at ward rounds, in collaboration with the various clinical departments. At the University of British Columbia, for instance, the final year students have five three-hour sessions in which cases of genetically determined diseases are presented, and discussed by a clinician, geneticist and other appropriate specialists. Thus the genetic aspects of the disease are presented as an integral part of the medical problem.

Postgraduate training in Medical Genetics can be considered at three levels — as part of the regular training in the various medical and surgical specialties, as more advanced training for those who wish to use genetics as an adjunct to their research and practice but do not wish to make it a full-time occupation, and as advanced training to qualify candidates for full-time research and teaching in Medical Genetics. I have time to mention only the last of these. A well-trained teacher and practitioner of Medical Genetics should be conversant with the genetics of man, of course, but also, to some degree, of other animals and of micro-organisms. He should be as familiar as possible with the tools of modern biological research. The report recommends that in those countries where the profession of a specialty is controlled, Medical Genetics should eventually be placed in the same position as are other specialties, and qualification as a specialist in Medical Genetics should be governed in the same way. (I am comforted by the thought that when this happens in Canada I shall either have retired, or as with other new specialties, I may be admitted as a charter member without examination.) A program, now in operation at Western Reserve, is outlined as a suitable course model, although not, of course, the only one. Finally, the Committee recommended that "in order to maintain a satisfactory teaching program in genetics, it would be highly desirable, as trained personnel become available, to have a chair or department of Medical Genetics at each medical school, with a staff to participate in the teaching and to create interest by their research activities." A word to the wise is sufficient. Go and see your Dean!

One thing the report did not discuss was the proper training for genetic counseling. The reason is obvious; once the statistical recurrence risk is established, counseling becomes an individual matter, and the approach depends largely on the counselor's personality and the situation at hand. How can you teach a person patience, wisdom, and compassion? Some aspects of medical school training can help to nourish these qualities, if they are there, and they

can grow with experience, if the counselor allows them to. It is held by some that psychiatric training is an asset in this regard, but apart from a brief exposure in medical school this is difficult for most of us to get. So about all we can do is try to be continually aware of the counselee as a person, and to maintain, humbly, a balance between scientific objectivity and sympathetic sensitivity. I am repeatedly struck by the profound impacts that a mutant gene can have on a family. There is no time to dwell on this, but I would like to mention one case I have been following for the past two years, which is often on my mind. A 27-year old girl came for advice because her mother and five of her six sisters all suffered from what she described as a "muscular wasting disease." Three of the sisters had already died from it, and her mother was severely crippled and barely able to walk. The girl, Sheila, suspected that this might be an inherited disease and was naturally worried about her chances of getting it or transmitting it to her children. She had avoided any prospects of marriage for this reason. Repeated efforts to find out what the disease was had been fruitless. Her family doctor, for instance, had told her flatly that it was not her concern and to forget about it. (Why?) I offered to look into it and made an appointment for another interview in three months time. After considerable correspondence, and some difficulty in getting release of information from certain hospitals, it became clear that the disease was Huntington's chorea, and there was evidence of dominant inheritance through the mother's father who had died, supposedly unaffected, at age 34, of alcoholism.

So Sheila had a little less than a 50:50 chance of developing it herself. What should I tell her? Apparently her physician thought it best she should not know, and I wondered myself whether I would want to know if I were in the same position. One geneticist I talked to about this stated definitely that he would prefer not to know. But Sheila was an intelligent girl who had been trying hard to find out. I felt her spirit was sturdy, and that she would never be satisfied just to go on wondering. So, in the course of almost a full morning, I described the situation to her. She was visibly shaken, but courageous.

We discussed the early signs, the possible value of an EEG, and many other things, including the relation of God to a situation like this. A month later she wrote me a letter thanking me for telling her, saying that although it was rough at first, it was certainly best to know the score, and very sweetly adding how difficult it must have been for me to tell her (which it certainly was). The following year she came in again "just to talk," but it was soon clear that she was worried about the early signs of the disease, and especially a little twitching of the muscle of her right arm. There is no doubt that fear of a disease can produce symptoms. Imagine what it is like to know, from watching a loved one develop the disease, exactly what the early signs are, and to wonder, with each minor memory lapse, each slip of the tongue, each accidental stumble, whether this is it. I referred Sheila to a neuropsychiatrist who specialized in organic brain disease, and after a thorough examination, completely negative, she was reassured and her twitches disappeared.

Over this period she had looked further into her family situation, and found that two of her first cousins were developing suspicious symptoms. She described the tragic personality changes that had occurred in her mother and

aunts, who became pathologically suspicious, terribly stubborn, and subject to frightful temper tantrums. The husbands of all four married aunts had deserted their wives, because life was such hell living with them. Only Sheila's father had stood by his wife, and she didn't know how long he would last. Furthermore, almost all the daughters of her four affected married aunts had married before they were twenty, in order to get away from their homes as soon as possible, with the dysgenic result that they will probably have more children than they otherwise would have.

This summer Sheila came in for another chat. Her mother can hardly walk, her speech is unintelligible, and she is terribly difficult to live with, but not crippled enough to be hospitalized. Sheila said she was enjoying life, outside the home, but then she burst out and said what a block this has been to her all her life, how bitterly she has fought it, how her father had brought her up to be strong ("All the Giffords are strong, and you're a Gifford, not a Pearson," he would say); how she is always thinking about it. "I hate this thing," she said. "I hate what it does to the person who has it and to everyone around her, and what it's done to me." She would like to marry and have children, but she's not a gambler — and even if she found a man who would be willing to adopt his children, she feared what she would do to him if she developed the disease. This is a great tragedy, because Sheila is the sort of person who would make a good wife and mother — and perhaps she doesn't carry the gene at all. I've told you about her to emphasize how much can be involved in the question of a mutant gene besides the statistical fact of a 50:50 segregation. What Sheila needed was moral support. Others need more practical aid. Let us not be content with providing risk rates and sympathy. There are cases that need to be referred to a gynecologist, others to a priest, others to a psychiatrist, and others to a social service worker or clinic that can ensure a regular family follow-up.

Counseling has its lighter moments too — for instance, the young Scots girl married to a Greek man whose first baby was born with an unusual combination of malformations. I still don't know exactly what it is, but the father's brother, who had married a relative, had had two similarly affected children. I discussed the recurrence risk with her as well as I could in our state of ignorance, and made a note in the chart that I would like to know the outcome of future pregnancies. Last year the hospital called to say there was a new baby, and would I come and examine it. She was beautifully normal, so I went in to congratulate the mother. While chatting with her, I became aware that she did not seem at all surprised that the baby was normal, but had a complacent look about her and an underlying gleam of amusement in her eye. On impulse I asked her if she had any secrets to tell me. She laughed and said "I think you already know." This is one method of coping with genetic problems which is, from a practical point of view, comparable to AID — artificial insemination donor. I call it NID — natural insemination donor, who, in this case, had been carefully chosen to resemble the husband in his physical features!

So there are many aspects of being a Medical Geneticist — discouraging, frustrating, bewildering, amusing, exciting, rewarding, and always challenging. It's a good job.

ADDENDUM

It would be a nice tradition for each President to make some sort of bequest to forward the work of the Society. The following contribution is therefore presented in readiness for the day when it will be possible to change the genetic constitution of man by DNA transformation. If the Society is on its toes it will patent the process, corner the market on human DNA, and conduct a vigorous advertising campaign, which will of course require a jingle for the TV commercial. This one is sung to the tune of "Smiles."

There are genes that make you happy
 There are genes that make you blue¹
 There are genes that tell you who's your father²
 And how you'll rate on your I.Q.
 There are genes that make your blood clot quickly
 And genes that tell how much you'll weigh³
 But if you don't like the genes you're born with
 TRY A.S.H.G. D.N.A.⁴

¹Congenital methemoglobinemia, for instance.

²Poetic license; actually, of course, they can usually only tell who's *not* your father.

³If you don't make a pig of yourself.

⁴Copyright pending.

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The Satellited Chromosomes of Man with Reference to the Marfan Syndrome

STANLEY D. HANDMAKER¹

*Division of Medical Genetics, Department of Medicine,
Johns Hopkins University School of Medicine
Baltimore, Maryland*

A WELL-DOCUMENTED feature of chromosome satellites in other organisms is the variation in size throughout a normal population. Heteromorphism of the human satellited chromosomes was first recorded by Tjio and Puck (1958), who detected three examples of heterozygosity in the size of the satellites in two individuals. No significance was attached to the variation in satellite size. Later, Tjio, Puck and Robinson (1960) reported two familial cases of the Marfan syndrome, in each of whom one chromosome bore a satellite enlarged to a degree greater than hitherto described. The authors postulated that enlarged satellites might be associated with familial cases of the Marfan syndrome.

It is the purpose of the present communication to present evidence demonstrating a lack of correlation between enlarged satellites and the Marfan syndrome.

MATERIALS AND METHODS

Chromosome analyses were made on eight patients with the Marfan syndrome and four unaffected relatives.

The chromosome studies were made on preparation from short-term cultures of white blood cells from the peripheral blood. The technique employed is based on that described by Moorhead, Nowell, Mellman, Battips and Hungerford (1960).

From each individual, 10-30 cc. of peripheral blood was drawn into a heparinized syringe and transferred to sterile screw-top tubes to which 'Bacto-Phytohemagglutinin' (Difco) was added (0.25 cc./10 cc. blood). The blood was refrigerated at 4°C. for from one to seven hours, then centrifuged at 300-400 r.p.m. for 10 minutes, and the supernatant and part of the buffy coat was decanted into sterile, two ounce prescription bottles containing T.C. medium 199 (Difco) (5-7 cc. medium /2-3 cc. serum and white blood cells). The cultures were refrigerated (4°C.) overnight and then incubated at 37°C. for 66-78 hours.

Deacetylmethyl colchicine ("Colcemide", Ciba) was added to each culture for the last 1-3 hours of incubation, at a final concentration of 2 μ g./ml. The cultures were decanted into centrifuge tubes and centrifuged at 800-1,000 r.p.m. for 5-10 minutes, and the supernatant was removed. Hypotonic sodium citrate solution (1.12 per cent) (7-10 cc.) was added to the residue in each tube, and they were incubated for 15 minutes at 37°C. The tubes were again centrifuged at 800-1,000 r.p.m. for 5-10 minutes, and the supernatant was discarded. The cells were fixed in cold 50 per cent acetic acid (7-10 cc.) and centrifuged at 800-1,000 r.p.m. for 5-10 minutes. All of the supernatant was discarded except for a few drops, in which the cells were resuspended. A drop of the cell

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¹Present Address: Albert Einstein College of Medicine, New York, New York.

TABLE 1. CLINICAL DATA ON EIGHT PATIENTS WITH MARFAN SYNDROME

Patient J.H.H. History No.	Age at Study	Sex Race	Clinical Abnormalities			Family History
			Eye	Skeleton	Cardiovascular System	
L.T., 22 11 83	30	Female Negro	Ectopia lentis; retinal detach- ments.	Typical arachnodactyly	None as yet	Father died at 39 yrs., autopsy confirmed dissecting aneurysm of aorta. (See Fig. 13, p. 59, McKusick, 1960a.)
R.C., 78 33 40	12	Male White	Ectopia lentis	Typical arachnodactyly kyphoscoliosis	None as yet	Father had ectopia lentis and died at 37 yrs. during operation for chronic dissec- tion of aorta; the paternal grandmother and a paternal aunt had well-confirmed Marfan syndrome. (see Fig. 17, pp. 72- 74, McKusick, 1960a.)
S.S., 71 96 04	12	Male Negro	Ectopia lentis	Typical arachnodactyly	Aortic diastolic murmur	Mother, an aunt and uncle, a half aunt, a cousin, and almost certainly the mater- nal grandmother were affected. (See Fig. 18, pp. 75-76, McKusick, 1960a.)
A.C., 59 38 33	16	Female White	Ectopia lentis	Typical arachnodactyly	None as yet	Father (E.C., 283314) and brother (R. C., 441759) are affected. Paternal uncle probably died of cardiac complications of Marfan syndrome. (See Fig. 20, p. 76, McKusick, 1960a.)
E.C., 28 22 14	37	Male White	Ectopia lentis	Moderate arachnodactyly chest deformity	None as yet	Father of A. C., 59 38 33
R.C., 44 17 59	14	Male White	Ectopia lentis	Moderate arachnodactyly	None as yet	Brother of A.C., 59 38 33
M.D., A5 99 49	15	Male White	Ectopia lentis	Marked arachnodactyly loose-jointedness, etc.	None as yet	Presumably sporadic case; one sib and both parents normal. (See Figs. 4A, 4B, 5, and 10A, p. 46 ff, McKusick, 1960a.)
V.W., 96 75 67	7	Female Negro	Ectopia lentis	Typical arachnodactyly	None as yet	Presumably sporadic case; 11 sibs and both parents are ostensibly unaffected.

suspension was pipetted onto each of several clean slides, and smears were made by the routine blood-smear method. The slides were air-dried quickly and then stained in aceto-orcein (2 per cent orcein in 65 per cent acetic acid), dehydrated, cleared, and mounted in balsam. The smears were scanned under low-power objective for metaphase plates suitable for analysis which were then examined under oil-immersion lens.

ANALYTICAL PROCEDURE AND RESULTS

Chromosome studies on eight patients with the Marfan syndrome and four normal relatives were made to determine whether any correlation exists between the Marfan syndrome and a visible chromosomal abnormality. Of the eight Marfan cases, six were definitely familial. Relevant diagnostic information is given in table 1.

At least 10 metaphase plates of each individual were selected for photography, and idiograms were prepared from prints enlarged to X4000 magnification. None of the idiograms of either Marfan cases or normal family members displayed any obvious translocations or other gross chromosomal aberrations. Chromosome counts were made on 64 unselected cells from the first familial Marfan case studied (L.T.). The modal number was clearly 46, and there was no evidence of mosaicism (table 2).

TABLE 2. CHROMOSOME COUNTS IN 64 CELLS OF A FAMILIAL CASE OF THE MARFAN SYNDROME

Chromosome count	<	43	44	45	46	47	>
Number of cells	6	6	1	5	44	1	1

Heterozygosity in the size of the satellite was clearly present in five of the Marfan patients, of whom three were in the same family. In the affected father (E.C. 282214), the enlarged satellite is on one chromosome in group VII (21-22) (Fig. 1). The affected daughter (A.C. 593833) has enlarged satellites on one chromosome in group IV (13-15) and one chromosome in group VII, the latter being the more prominent (Fig. 2). The brother of A.C. is also affected (R.C. 441759) and he has prominent satellites on one chromosome in group IV and one in group VII. However, the mother (H.C.), who is clearly unaffected, also has enlarged satellites on one chromosome in group IV and one in group VII (Fig. 3). The satellite on the group VII chromosome is the more prominent one, and it is clearly as prominent as that of any of the affected members of the family.

DISCUSSION

The Marfan syndrome is a heritable disorder of connective tissue, usually displaying autosomal dominant transmission (McKusick, 1960a). The earlier chromosome studies of Marfan cases by several investigators had revealed no detectable chromosomal aberration (Tjio, Puck and Robinson, 1959; Ford, 1960; Böök, Fracaro and Lindsten, cited by Ford). However, in reporting two

further cases, Tjio, Puck, and Robinson (1960) stated that their original case lacked a familial history of the disease, whereas the pedigrees of the two additional cases were both consistent with autosomal dominant transmission. In describing the results of chromosome studies of these two patients they noted the following: (1) The first patient possessed a "tremendously enlarged" satellite on one member of pair number 21. (2) In the second patient, the enlarged satellite was on one member of pair number 13. (3) In both cases, the structure was consistent in all the cells examined. (4) The satellite, in both cases, was clearly larger than any satellite seen in human subjects, not excluding those previously described as enlarged (Tjio and Puck 1958a). No studies of other affected or unaffected members of the families were reported.

Tjio, Puck, and Robinson (1960) stated, "While the present data do not

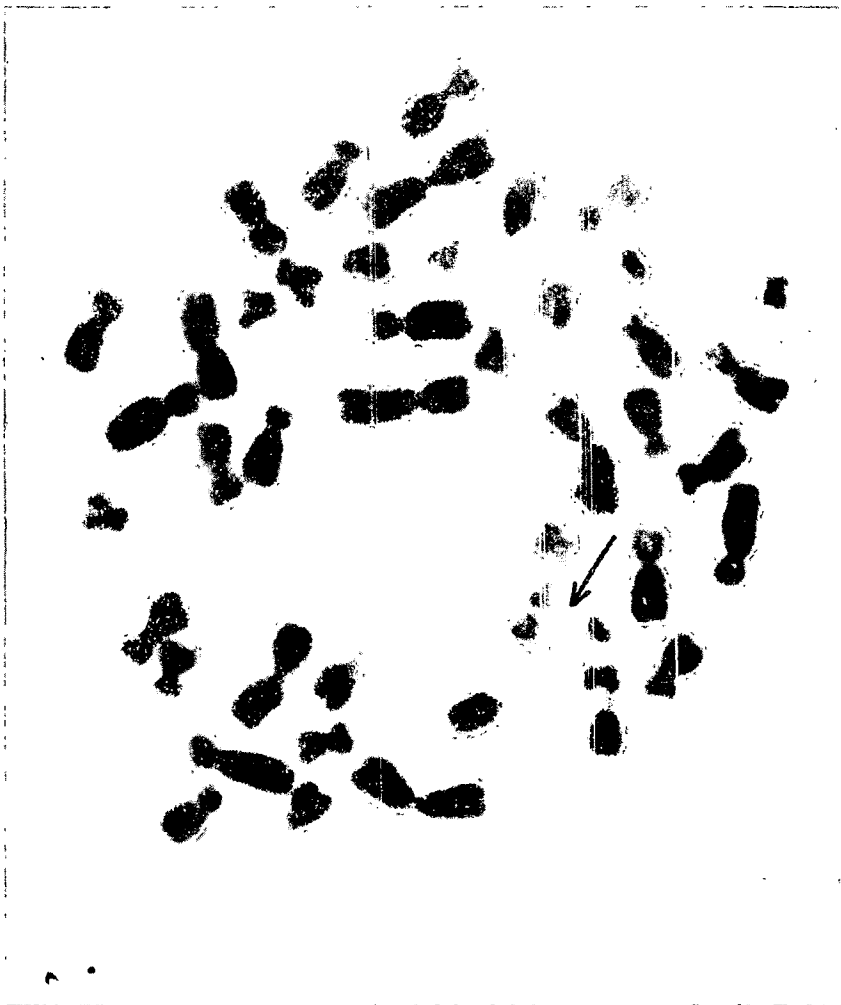


FIG. 1. A metaphase plate of E. C., 282214, a patient with the Marfan syndrome. E.C. is the father of A.C., 593833, and R.C., 441759 (table 1). The arrow indicates the prominent satellite on one chromosome in group VII (21-22).

prove the given abnormalities to be the underlying cause of Marfan's syndrome, a relationship is definitely suggested, since in the analysis of chromosomes from more than 30 human subjects, satellites enlarged to the degree here described have been found in only those two patients who exhibit this particular disease, and in the form which displays a dominant genetic pattern. The heterozygosity of the defect in each case is in accord with the mode of inheritance. It is unexpected, however, that the structural defect obtained in these two cases should involve different chromosomes, and a causal connection between this aberration and the given clinical condition can only be maintained on the assumption that damage to either satellite can cause similar cellular derangement."

While the cytological findings of enlarged satellites are unquestioned, any causal relationship between enlarged satellites and the Marfan syndrome is contradicted by the following evidence: (1) As McKusick (1960b) noted, "It is by no means certain that the two cases with satellite peculiarities had the true Marfan syndrome." (2) Such enlarged satellites have been seen in many individuals who obviously do not have the Marfan syndrome. (Buckton and Harnden, personal communication; Cooper and Hirschhorn, 1962; Ellis and Penrose, 1961; Ferguson-Smith, personal communication.) (3) Chromosome studies of eight Marfan patients, six definitely familial, are reported in this communication, and of these cases none possesses a satellite enlarged to a degree greater than that observed in unaffected relatives or that often seen in individuals who display no features of the Marfan syndrome.

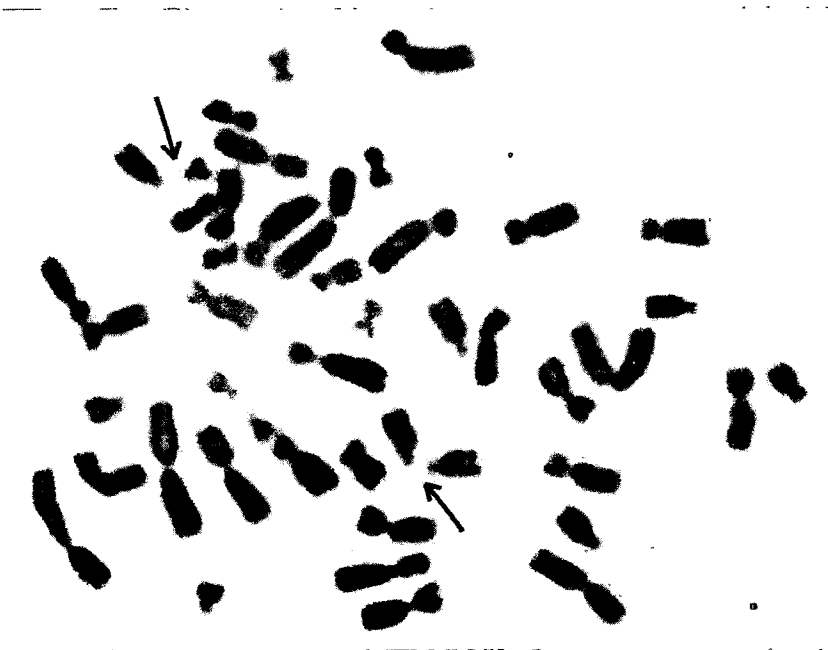


FIG. 2. A metaphase plate of A.C., 593833, a patient with the Marfan syndrome. A.C. is the daughter of H.C. and E.C. 282214. The arrows indicate the prominent satellites on one chromosome in group IV (13-15) and one chromosome in group VII (21-22).

Thus far, there is no firm basis for considering that a correlation exists between enlarged satellites and any pathological condition. One can only conclude that the presence of enlarged satellites in the two familial Marfan cases reported by Tjio, Puck, and Robinson (1960) was fortuitous.

Kallen and Levan (1962) recently reported that chromosomes 21 and 22 in four Marfan patients were relatively shorter than those of ten normal control subjects. However, since the control subjects were not relatives of the Marfan patients, it is not possible to determine whether the differences in chromosome length are associated with the Marfan syndrome or are simply normal familial variations unrelated to the disorder.

In human genetic studies, the importance of an enlarged satellite lies in its use as a cytogenetic marker. By distinguishing one chromosome of a pair

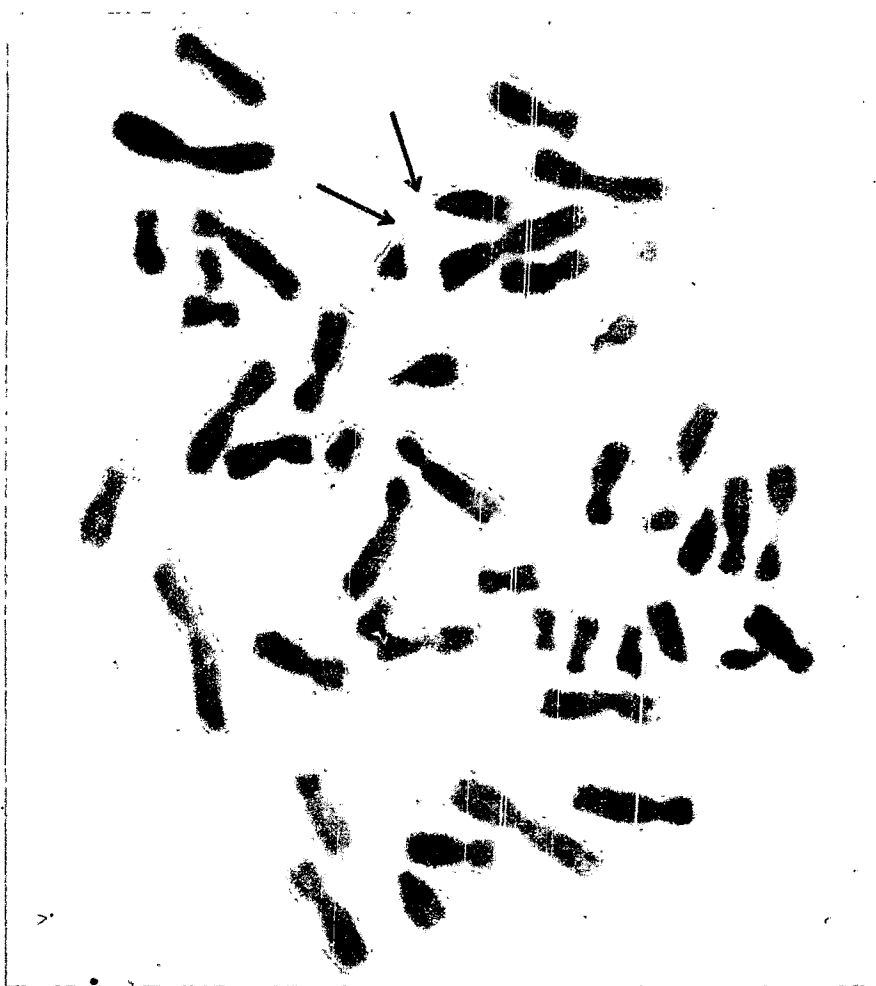


FIG. 3. A metaphase plate of H.C., unaffected. H.C. is the wife of E.C., 282214, and the mother of A.C., 593833, and R.C., 441759. The arrows indicate the prominent satellites on one chromosome in group IV (13-15) and one chromosome in group VII (21-22).

from its homologue on the basis of differential satellite size, one can often determine which member of the pair comes from which parent. As a consequence of this, family studies can be made which could enable one to determine whether a known gene segregates independently of a particular chromosome in a given family. If the gene does not segregate independently of the satellite-marked chromosome in one family, several such families must then be studied for statistically significant results. If the gene does segregate independently of the satellite peculiarity, then the gene may be on a separate chromosome or may be located so far from the satellited end of the chromosome that frequent crossing over occurs. The reasoning here is identical to that in all linkage analysis.

Thus far, there have been two attempts to locate an autosomal locus on a specific chromosome by combining studies of blood groups and serum proteins with the use of enlarged satellites as cytogenetic markers (Ellis and Penrose, 1961; Cooper and Hirschhorn, 1962). In one family there was evidence of repulsion between Le (a+) and the chromosome bearing an enlarged satellite (Ellis and Penrose, 1961).

A major problem confronting the human geneticist is the detection of genetic carriers, "those individuals who will never in their lifetime develop the disease which they transmit, and those who may at some date subsequent to the original examination be characterized by the typical signs and symptoms of the disorder in question" (Neel, 1949). Theoretically, a most promising approach to this problem has been the attempt to establish genetic linkage between a given deleterious gene and a nearby locus having two or more recognizable allelic genes, each with relatively high frequency. Several such linkage groups have been ascertained, but only in the case of the X-chromosome have linkage groups been assigned to a specific chromosome. By combining linkage studies with the use of cytogenetic markers such as satellite enlargement, the possibility exists of locating a known autosomal linkage group on a particular chromosome.

SUMMARY

The somatic metaphase chromosomes of eight patients with the Marfan syndrome and four normal family members were studied. Heterozygosity in the size of the satellite is clearly present in five of the Marfan cases; however, none of these satellites is more prominent than those of either the unaffected relatives studied or other individuals without stigmata of the Marfan syndrome, indicating a lack of correlation between enlarged satellites and the Marfan syndrome.

ACKNOWLEDGMENTS

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Application of the Gc System in Paternity Cases

JAN HIRSCHFELD AND AAGE HEIKEN

State Institute for Blood Group Serology, Stockholm, Sweden

THE GROUP-SPECIFIC components (Gc) consist of electrophoretically different but immunologically identical α_2 -globulins, which allow a division of human sera into three main types by means of immuno-electrophoresis (Hirschfeld, 1959). These different types are, in analogy with the haptoglobin types, called Gc 1-1, Gc 2-1, and Gc 2-2. Sera belonging to Gc 1-1 and Gc 2-2 are characterized by a fast and a slow group-specific component, respectively; whereas sera belonging to Gc 2-1 contain both the fast and the slow components (Fig. 1). Genetic studies have confirmed the inheritance of the Gc system as an autosomal two-allele system without dominance, where Gc 1-1 and Gc 2-2 are the homozygotes and Gc 2-1 is the heterozygote (Hirschfeld, Jonsson and Rasmuson, 1960).

So far, no relation of the Gc system with other blood and serum groups has been found (Hirschfeld and Beckman, 1961; Hirschfeld, 1962). Recent works seem to imply a connection between the Gc system, as demonstrated in immuno-electrophoresis, and the post-albumins, as demonstrated by starch gel electrophoresis. The post-albumins do, however, seem to contain several other components (Schultze, Biel, Haupt and Heide, 1962 a, b), some of which might vary independently of each other (Tombs, James and MacLagan, 1961).

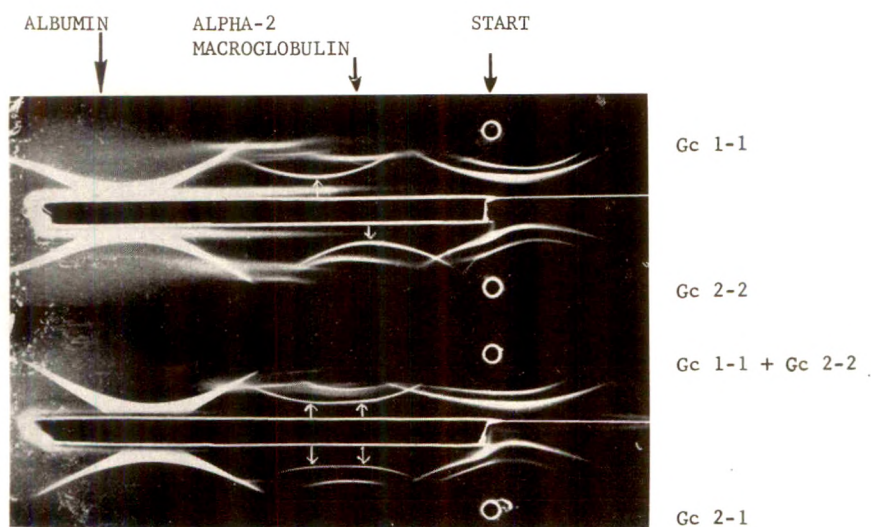


FIG. 1. Immuno-electrophoretic patterns of the three main Gc types and a mixture of the homozygotes in equal amounts. The anode is to the left. The Gc precipitates are indicated by arrows.

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Therefore, in the following, the same nomenclature will be used as originally given to the group-specific components by Hirschfeld and Beckman (1960).

The purpose of this paper is to describe and discuss the prefatory results obtained by the use of the Gc system in paternity cases at the State Institute for Blood Group Serology in Stockholm.

MATERIAL AND METHODS

The Gc system is demonstrated by means of the immuno-electrophoretical method of Grabar and Williams (1953) with a modified technique described in detail by Hirschfeld (1960).

The present investigation includes sera arriving from the entire country in connection with paternity cases. These serum samples were subjected to immuno-electrophoresis in two independent experiments or until two clear-cut results were obtained. The Gc determinations were carried out without knowledge of previous typing results or relation of the particular serum to other sera investigated. From part of this material, 67 cases consisting of mother, child and one alleged father, and 75 cases consisting of mother, child and two possible fathers were extracted. From the remaining material, a sample of unrelated adults was included in the analysis. The three categories will be referred to as "one-man cases," "two-man cases," and "incomplete cases," respectively.

RESULTS AND CONCLUSIONS

Frequency of the Gc Genes.—The typing results of the material consisting of 515 unrelated, adult individuals are given in table 1. As the difference between this sample and two previously investigated samples including 1,162 (Hirschfeld and Beckman, 1960) and 582 (Hirschfeld, 1962) persons, respectively, was insignificant ($0.2 > P > 0.1$), the pooled number consisting of 2,259 unrelated individuals was used in the calculation of the gene frequencies, which are for $Gc^1=0.7459$ and for $Gc^2=0.2541$. These values differ only slightly from those earlier obtained for the Swedish population. The expected frequencies of the three genotypes, which will be used in the following tables, are $Gc^1/Gc^1=55.637$ per cent, $Gc^2/Gc^1=37.906$ per cent, and $Gc^2/Gc^2=6.456$ per cent.

Inheritance of the Gc System. The 142 mother-child combinations of the present investigation are given in table 2. A close agreement between observed and expected values was obtained ($0.2 > P > 0.1$). Thus, up to the present, 1,508 mother-child combinations and 567 families with 1,469 children have been Gc-typed without finding any exceptions from the genetic theory (Hirsch-

TABLE 1. DISTRIBUTION OF GC TYPES IN 515 UNRELATED SWEDES

Type	Males			Females			Males and females			Total	
	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2	No.	%
Gc 1-1	116	120.7	0.18	81	79.0	0.05	97	86.8	1.20	294	57.09
Gc 2-1	88	82.3	0.39	54	53.8	0.00	47	59.1	2.48	189	36.70
Gc 2-2	13	14.0	0.07	7	9.2	0.53	12	10.1	0.36	32	6.21
Total	217	217.0	0.64	142	142.0	0.58	156	156.0	4.04	515	100.00

feld *et al.*, 1960; Cleve and Bearn, 1961 a, b; Baitsch, 1962; Hess and Bütler, 1962; Hirschfeld, 1962; Hirschfeld and Lunell, 1962; Jenssen, 1962; Neerström, 1962; Reinskou and Mohr, 1962).

Exclusion Possibilities on the Basis of the Gc System. In table 3, the expected frequencies of mother-child-alleged father combinations are given assuming no relation between the mother-child combination and the alleged father. On the basis of these figures, the expected frequencies of the different combinations leading to an exclusion in paternity tests are calculated and presented in table 4. The sum of these frequencies is 15.36 per cent, which is thus the theoretical exclusion rate for the Gc system.

In the 142 cases of disputed paternity, 12 exclusions were found. In 10 of these, the alleged father was of the type Gc 1-1, the mother and child being Gc 1-1 and Gc 2-1, respectively. The difference between the Gc system and the

TABLE 2. GC TYPES IN 142 MOTHER-CHILD COMBINATIONS

Mother/child Combinations	One-man Cases			Two-man Cases			Total		
	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2
Gc 1-1/Gc 1-1	26	27.8	0.12	34	31.1	0.27	60	58.9	0.02
Gc 1-1/Gc 2-1	9	9.5	0.03	12	10.6	0.18	21	20.1	0.04
Gc 1-1/Gc 2-2	0	0.0	—	0	0.0	—	0	0.0	—
Gc 2-1/Gc 1-1	12	9.5	0.66	9	10.6	0.24	21	20.1	0.04
Gc 2-1/Gc 2-1	16	12.8	0.80	16	14.3	0.20	32	27.1	0.89
Gc 2-1/Gc 2-2	1	3.2	1.51	0	3.6	3.60	1	6.8	4.95
Gc 2-2/Gc 1-1	0	0.0	—	0	0.0	—	0	0.0	—
Gc 2-2/Gc 2-1	3	3.2	0.01	3	3.6	0.10	6	6.8	0.09
Gc 2-2/Gc 2-2	0	1.1	1.10	1	1.2	0.03	1	2.3	0.73
Total	67	67.1	4.23	75	75.0	4.62	142	142.1	6.76

TABLE 3. EXPECTED FREQUENCIES OF MOTHER-CHILD-MAN COMBINATIONS PROVIDED NO RELATION EXISTS BETWEEN MOTHER-CHILD AND ALLEGED FATHER

Mother/child Combinations	Frequency, %	Phenotype of Alleged Father		
		Gc 1-1	Gc 2-1	Gc 2-2
Gc 1-1/Gc 1-1	41.51	23.10	15.74	2.68
Gc 1-1/Gc 2-1	14.14	7.87	5.36	0.91
Gc 2-1/Gc 1-1	14.14	7.87	5.36	0.91
Gc 2-1/Gc 2-1	18.95	10.54	7.18	1.22
Gc 2-1/Gc 2-2	4.82	2.68	1.83	0.31
Gc 2-2/Gc 2-1	4.82	2.68	1.83	0.31
Gc 2-2/Gc 2-2	1.64	0.91	0.62	0.11
Total	100.02	55.65	37.92	6.45

TABLE 4. THEORETICAL RATES OF EXCLUSION

Mother	Phenotype		Exclusion Rate %
	Child	Excluded father	
Gc 1-1	Gc 2-1	Gc 1-1	7.87
Gc 1-1	Gc 1-1	Gc 2-2	2.68
Gc 2-1	Gc 2-2	Gc 1-1	2.68
Gc 2-1	Gc 1-1	Gc 2-2	0.91
Gc 2-2	Gc 2-2	Gc 1-1	0.91
Gc 2-2	Gc 2-1	Gc 2-2	0.31
Total			15.36

Hp system regarding the ratio between theoretical and observed exclusions was not significant (table 5).

TABLE 5. COMPARISON OF GROUP-SPECIFIC COMPONENTS (Gc) AND HAPTOGLOBINS (Hp) AS TO THEORETICAL AND OBSERVED EXCLUSION RATES

	Gc	Hp
Theoretical exclusion rate	15.36 %	18.02 %
<i>One-man cases</i>		
observed exclusion rate	3/67 = 4.48 %	3/60 = 5.00 %
ratio observed/theoretical exclusion rate	0.29	0.28
<i>Two-man cases</i>		
observed exclusion rate	9/150 = 6.00 %	16/162 = 9.88 %
ratio observed/theoretical rate	0.39	0.55

SUMMARY

Data for the Gc determinations in 142 cases of disputed paternity are given. On the basis of the gene frequencies for 2,259 unrelated Swedes, the theoretical exclusion rate could be calculated to be 15.36 per cent. Regarding its high degree of biological and technical reliability, it may be concluded that the Gc system is a valuable addition to the genetical markers already in use in paternity cases.

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The Demonstration of Secondary Constrictions in Human Chromosomes by Means of a New Technique

MASAO S. SASAKI AND SAJIRO MAKINO

Zoological Institute, Kokkaido University, Sapporo, Japan

RECENT ADVANCES in cytological technique and methods for culturing mammalian tissues have greatly facilitated the detailed study of the structure of mammalian and human chromosomes. Through the application of cytogenetic techniques to human material many cases of abnormal chromosome patterns have been discovered which were found to be associated with specific human diseases. At the meeting in Denver (April 1960) it was agreed to classify the human complement into seven groups of autosomes and the sex chromosomes on the basis of total length and arm ratio (Robinson, 1961). It has been stated, however, that the arranging of chromosomes according to the Denver system is more ambiguous than it seems (Patau, 1960, 1961).

Students of human chromosomes are aware that secondary constrictions, if shown to be consistent features of certain chromosomes, would be useful criteria for identifying individual chromosomes in karyotype analysis. The presence of secondary constrictions has been briefly described in certain chromosomes of group 6-12 (Patau, 1961; Patau, Therman, Inhorn, Smith, and Ruess, 1961; De la Chapelle, 1961; Muldal and Ockey, 1961). However, there remained a need for special methods that would demonstrate secondary constrictions more clearly than they are seen in ordinary preparations. Such methods would be of great value for individual recognition of human chromosomes, particularly in relating chromosome abnormalities to various disorders, especially certain genetic diseases and cancer. While working on a chromosome survey in human populations, one of us (M.S.S.) came across a simple new technique for the demonstration of secondary constrictions. Application of this method to several embryonic tissues frequently revealed secondary constrictions, although there may still be some doubt whether these are comparable to the secondary constrictions of plant chromosomes. It seems to the authors that publication of this technique and the results obtained may be useful, even though they are not wholly consistent.

MATERIALS AND METHODS

Culture Procedures

Cultures were derived from skin or lung obtained from 11 legally aborted embryos three to four months old. Small pieces of tissue were rinsed with Rinaldini's physiological salt

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solution, minced with scissors, and treated with a 0.2 per cent trypsin solution. The tissues were then washed with two changes of culture medium. The resulting cell suspension was explanted in tissue-culture flasks, TD-15 or TD-40, and incubated at 37°C. The medium of McCoy, Maxwell, and Neuman (1956) was used as modified by Sasaki and Makino (1962). The cytological work was done with cells from primary cultures derived from six individuals and cells from two other individuals after subculturing for 30 to 37 generations.

Techniques for Demonstrating Secondary Constrictions

The new method for detection or exaggeration of secondary constrictions consists of eight steps:

1. Wash the cells in three changes of calcium-free medium (the above specified medium without calcium chloride) prewarmed to 37°C.
2. Incubate the cells in calcium-free medium at 37°C. for about six hours.
3. Treat the cells with colchicine (50×10^{-8} M) for one and one-half hours at 37°C.
4. Trypsinize the cultures with a 0.2 per cent solution of trypsin.
5. Centrifuge the cell suspension for about five minutes at 1,200 rpm.
6. Remove the supernatant fluid, place the cells in distilled water on slides, and leave them there for 20 to 30 minutes.
7. Squash the cells in acetic dahlia solution (0.75 Gm. of dahlia violet dissolved in 100 ml of 30 per cent acetic acid).
8. Seal the cover slip, for instance with the balsam-paraffin mixture of Makino and Nishimura (1952).

RESULTS

Metaphase chromosomes were examined in slides prepared as described above. The secondary constrictions made visible in certain chromosomes by the new technique generally appeared as more or less pronounced faintly stained gaps (Fig. 1-4). Table 1 shows the frequency of manifestation of the secondary constriction of chromosome No. 1 in cells cultured in the Ca-free medium. No significant difference was found in this frequency between primary cultures and long-term cultures.

One specimen (No. 5), in calcium-free medium, was checked at intervals

TABLE 1. FREQUENCY, IN PER CENT, OF CELLS WITH SECONDARY CONSTRICTION ON CHROMOSOME NO. 1

Specimen			2.5	3.5	4	Hours 6	of 6.5	Ca-free 7	Culture 8	8.5	12	20
1	Primary culture	F Skin	—	—	—	—	—	—	65.2	—	—	—
2	Primary culture	M Skin	—	48.0	—	—	77.5	—	—	—	—	—
3	Primary culture	M Lung	—	—	—	100	—	—	—	—	—	—
4	Primary culture	M Lung	0	—	—	74.3	—	—	—	84.7	—	—
5	Primary culture	M Lung	—	8.3	—	81.8	—	71.6	47.1	—	65.5	—
6	Primary culture	F Skin	—	0	—	82.6	—	—	—	—	—	38.2
7	Primary culture	M Lung	—	—	13.3	65.7	—	—	—	100	—	—
8	Primary culture	M Lung	—	6.9	—	—	52.3	—	—	—	—	—
9	Primary culture	F Lung	—	—	—	—	—	—	—	—	74.3	—
0	Subculture-I	M Lung	—	—	—	—	—	—	—	—	—	—
	30th gener.		—	—	—	—	80.0	—	—	—	—	—
	31st gener.		—	22.1	—	—	—	—	—	83.2	—	—
	32nd gener.		—	—	—	—	—	58.0	—	—	—	—
	34th gener.		—	—	—	100	—	—	—	—	—	—
1	Subculture-II	F Lung	—	—	—	—	—	—	—	—	—	—
	35th gener.		—	—	—	92.2	—	—	—	—	—	—
	37th gener.		—	—	—	83.4	—	—	—	—	—	—

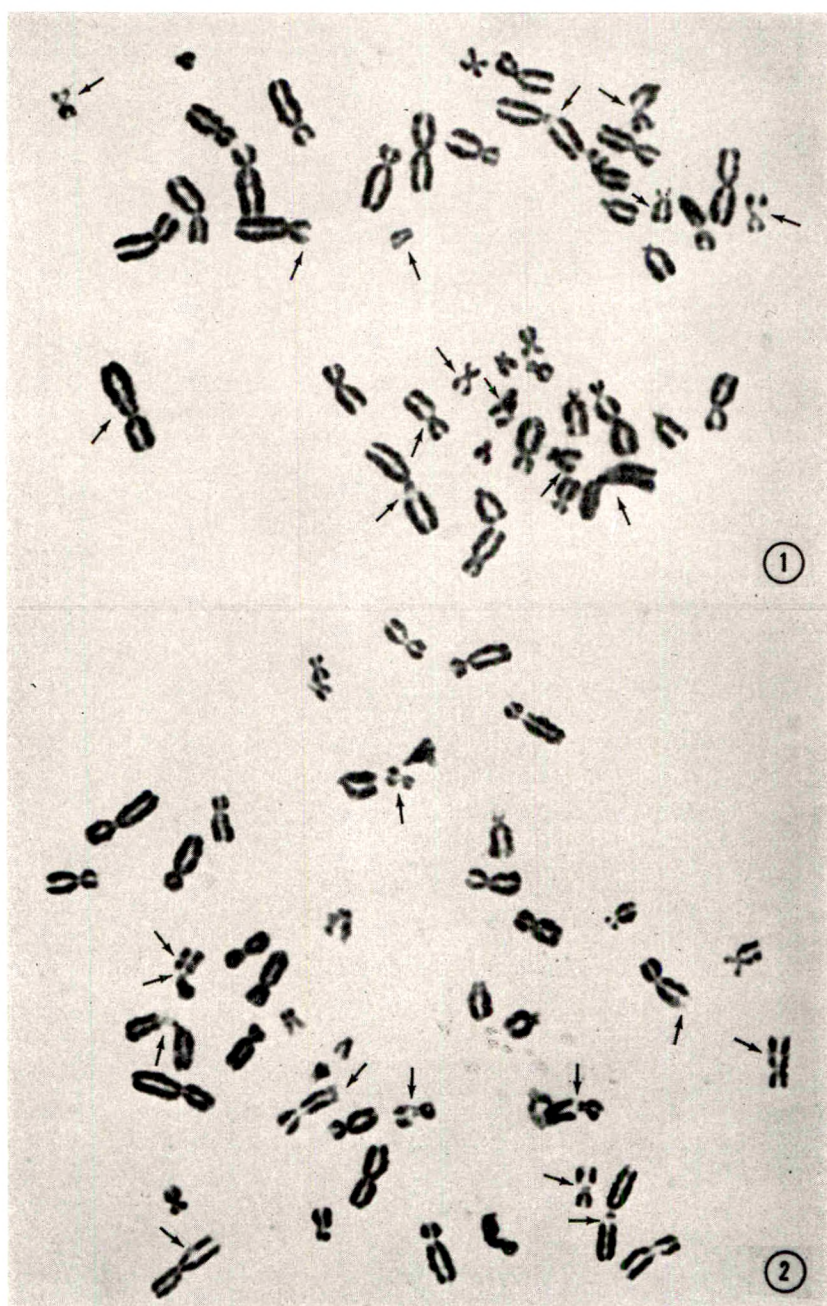


FIG. 1. Photomicrograph of a chromosome complement derived from a lung culture (male, 32nd subculture), 7 hours' Ca-free culture. Secondary constrictions are shown by arrows.

FIG. 2. Photomicrograph of a chromosome complement derived from a lung culture (female, primary culture), 12 hours' Ca-free culture. Secondary constrictions are shown by arrows.

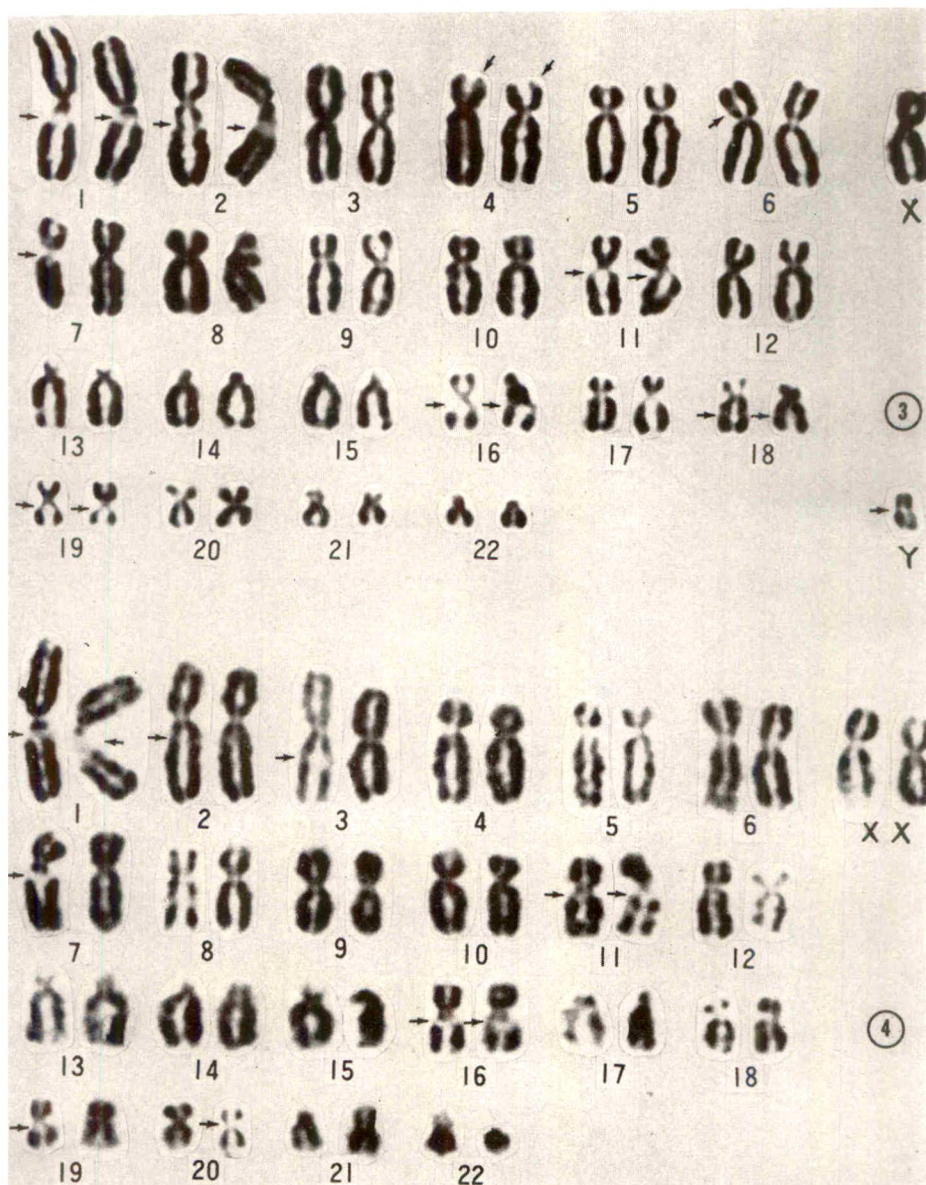


FIG. 3. Karyotype of a male complement, same cell as Fig. 1. Secondary constrictions are shown by arrows.

FIG. 4. Karyotype of a female complement, same cell as Fig. 2. Secondary constrictions are shown by arrows.

of one to two hours for mitotic rate, incidence of secondary constrictions in several chromosomes, and satellite association of chromosomes (table 2). After $3\frac{1}{2}$ to 5 hours in calcium-free medium, the cells showed a remarkable increase in mitotic rate, up to about 6 per cent. Generally such slides contained some sticky chromosomes. Good chromosome figures were obtained in samples taken after 6 to 8 hours in calcium-free medium. Secondary constrictions were gen-

TABLE 2. FREQUENCY OF SECONDARY CONSTRICTIONS IN SEVERAL CHROMOSOMES, MITOTIC INDEX, AND SATELLITE ASSOCIATION IN A PRIMARY CA-FREE CULTURE OF EMBRYONIC LUNG (MALE, NO. 5)

Hours of Ca-free Culture		0*	3.5	5	6	7	8	10	12	14	15.5	23.5	No. of Cells Observed
Incidence of secondary constrictions (% of cells)	Chromos. No.												
	1	—	8.3	44.5	81.8	71.6	47.1	44.8	65.5	66.7	69.7	19.1	
	2	—	—	—	26.3	28.6	29.4	7.9	10.3	11.1	18.3	9.5	
	3	—	—	—	15.8	9.5	11.8	2.6	6.9	—	8.7	—	
	4	—	—	—	13.2	19.0	5.9	—	13.4	—	11.2	—	
	7	—	—	—	42.1	42.8	17.6	7.9	31.0	11.1	11.2	9.5	
	11	—	—	—	47.5	71.6	47.0	21.1	55.2	44.5	44.5	27.6	32
	16	—	—	—	42.1	66.5	47.0	26.4	58.5	55.6	62.3	23.8	—
	18	—	—	—	39.5	32.3	34.3	15.8	20.6	—	—	9.5	
	19 and/or Y	—	—	—	23.7	28.6	17.6	7.9	27.6	33.2	8.7	—	
		—	—	5.4	42.1	52.4	53.4	26.4	51.7	44.5	44.5	27.6	
Mitotic index (%)		1.92	5.59	6.52	4.99	2.15	1.87	2.44	1.31	2.15	1.02	1.41	786 — 1562
Cells with sat. assoc. (%)		0	4.2	13.2	10.5	11.6	5.9	7.9	10.3	11.1	4.4	0	32 — 79

*In a culture with the normal medium.

ally seen more clearly after a longer time in calcium-free culture, but prolonged stay in this medium also decreased the mitotic rate.

The appearance and position of the secondary constrictions in specific chromosomes shall now be described.

Chromosomes of group 1-3. The longer arm of chromosome 1 was found to bear a distinct secondary constriction near the centromere. A high frequency of chromosome 1 with this constriction was observed in different cultures and at various intervals of growth in calcium-free medium (table 1). In metaphase chromosomes, the secondary constriction appeared typically as a pale-staining gap on both chromatids, the distal end of the gap lying at about one-third of the arm length from the centromere. Occasionally a tiny thickening of the chromatid was observed in the lightly staining gap (Fig. 5).

Chromosome 2 showed a secondary constriction on its long arm at a distance of about two-fifths from the centromere. The highest frequency of this secondary constriction was 28.6 per cent (table 2).

Chromosome 3 was found to carry a narrow gap on its longer arm at a distance of about three-fifths from the centromere. The constriction appeared with a frequency of 15.8 per cent (table 2).

Chromosomes of group 4-5. We have observed a secondary constriction on the short arm of what represents probably chromosome 4. It covered about one-third of the arm length from the distal end (Fig. 3). The frequency of this constriction was 19.0 per cent (table 2). This constriction may be useful for distinguishing the two chromosomes of this group.

Chromosomes of group 6-12. Chromosome 6 was found to have a narrow secondary constriction near the middle part of its short arm (Fig. 3). Its frequency was not determined. Chromosomes 7 and 11 were characterized each by a remarkable secondary constriction on their long arm with a frequency of approximately 40 per cent (table 2). The secondary constriction on the long arm of chromosome 7 extends from near the centromere to about one-third of the arm length (Fig. 3 and 4). It is interesting to note that this constriction was found with a very high frequency in one member of chromosome pair 7. Chromosome 11 revealed a distinct constriction on its long arm, appearing as a lightly staining gap from near the centromere to about one-third of the arm length.

Chromosomes of group 13-15. We have observed cases of lightly staining regions very close to the centromere in chromosomes belonging to this type. After the calcium-free treatment, the short arms of these chromosomes could not always be detected, but even on these chromosomes the satellites could be seen clearly.

Chromosomes of group 16-18. Chromosome 16 shows a very high frequency (42-62 per cent) of a remarkable secondary constriction on its long arm. This constriction extends from the centromere to the middle of the long arm (Fig. 3 and 4). Chromosome 18 was found to have a narrow constriction with low frequency in the middle of its long arm.

Chromosomes of group 19-20. Since these chromosomes are very similar in size and shape their identification is quite difficult. In some especially good preparations chromosome 19 showed a constriction on its long arm adjacent to

the centromere and chromosome 20 on its short arm, also close to the centromere (Fig. 3 and 4).

Chromosomes of group 21-22. Some chromosomes of this group showed a lightly staining region near the centromere.

X and Y chromosomes. The chromosome tentatively identified as X by its length and centromere position showed faintly staining gaps on its long arm. These were variable in number as well as in position. It is therefore difficult to characterize the X-chromosome on the basis of secondary constrictions. The Y-chromosome had very frequently a remarkable secondary constriction on the long arm. The distance of the distal end of this constriction from the centromere amounted to about three-fifths on the arm length. The constriction was seen in cells derived from each of the seven male embryos studied.

Notes on satellite association. At metaphase, about 10 per cent of the cells cultured for a short time in calcium-free medium showed, in addition to secondary constrictions, satellite association. Association between two satellited chromosomes was most frequent. Sometimes a satellited chromosome was found to be associated with the secondary constriction of chromosome 1 (Fig. 6-8).

DISCUSSION

Due largely to the work of Darlington and La Cour (1938), secondary constrictions have been used to a considerable extent in the karyotype analysis of plants. The differential staining of chromosomes has been related to a reduced supply of nucleic acids (Haga, 1958). Some investigators have also tried to

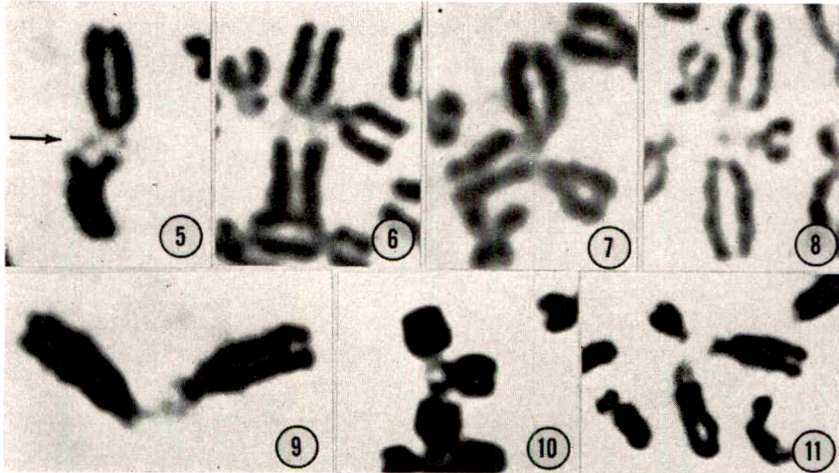


FIG. 5. Chromosome no. 1 showing tiny thickening of chromatids in the secondary constriction.

FIG. 6-7. Association of one of large acrocentric chromosomes with the secondary constriction of chromosome no. 1.

FIG. 8. Association of one of small acrocentric chromosomes with the secondary constriction of chromosome no. 1.

FIG. 9-11. Satellite associations in acrocentric chromosomes.

demonstrate secondary constrictions in mammalian chromosomes (Ohno and Kinosita, 1955; Gläss, 1956). In the Chinese hamster, Yerganian, Kato, Leonard, Gagnon and Grodzins (1960) observed a secondary constriction in one of the X-chromosomes, and Hsu and Somers (1961) proved the presence of a secondary constriction in the longest chromosome after treatment with 5-bromodeoxyuridine. In human chromosomes, Patau (1961) briefly mentioned a secondary constriction in an autosome of group 6-12. Patau *et al.* (1961) described the secondary constriction in chromosome 1. De la Chapelle (1961) and Muldal and Ockey (1961) published interesting reports on a larger chromosome with a secondary constriction in group 6-12 and suggested that this might be one of the X-chromosomes. Evidently, current knowledge on the secondary constrictions of human chromosomes is fragmentary.

We have shown in this paper that the short-term culture of cells in calcium-free medium causes secondary constrictions of human chromosomes to appear as achromatic gaps in specific loci of certain chromosomes. However, it remains to be shown that secondary constrictions demonstrated by this method are consistent enough to be of help in the identification of individual chromosomes in karyotype analysis. Presumably the constrictions correspond to regions conventionally known as heterochromatin, which had been considered to be genetically inactive. Calcium and magnesium are thought to have special significance in the metabolism of nuclear proteins and for the activation of many enzymes. Deficiency in calcium may cause unbalanced synthesis of RNA and protein, which have been shown to precede DNA synthesis (Taylor, 1960; Mirsky and Osawa, 1961), and thus cause the allocyclic behavior of the heterochromatin of the chromosomes. In this connection it is of interest that Tobias (1958) found marked allocyclus of chromosomes in animals fed on a poor diet, and suggested that a calcium-free diet might have affected DNA synthesis and thus caused allocyclus of the heterochromatin. This may result in the formation of achromatic gaps, which are generally referred to as secondary constrictions in metaphase chromosomes. The above interpretation is tentative; there may be other factors involved in the production of such achromatic gaps. Recently Makino and Sasaki (1961) noted an allocyclic condition of the Y-chromosome of man related to its heterochromatic nature.

Of particular interest is the behavior of the secondary constriction in chromosome 7. One member of this chromosome pair showed the secondary constriction in most cells studied regardless of the sex of the material. This reminds us of the findings of De la Chapelle (1961) and of Muldal and Ockey (1961), who suggested that a large element bearing a secondary constriction might be one of the X-chromosomes in man. Our findings suggest that the chromosome with the secondary constriction which was considered as a likely X element by these authors might be a member of the seventh pair. A final solution of this question requires further observations with sufficient material.

It was shown in the present study that after a short-term exposure of cells to calcium-free medium, satellite association occurs in a considerable number of cases. Ferguson-Smith and Handmaker (1961) and Ohno, Trujillo, Kaplan, and Kinosita (1961) have suggested that the acrocentric chromosomes displaying satellite association are involved in the organization of nucleoli. It seems

probable to us that the calcium-free medium affects the nucleoli, thus leaving groups of chromosomes with associated satellites. We further observed that chromosome 1 was sometimes associated through its secondary constriction with the satellites of some acrocentric chromosomes. Similar pictures were described by Shaw (1961) in leukocyte and fibroblast cultures of man. Schultz and St. Lawrence (1949), in a study on human pachytene chromosomes, stated that one of the longest chromosomes appeared to be a nucleolar organizing chromosome, with the nucleolar organizer near its middle. It seems thus likely that some of the chromosomes with secondary constrictions, for instance chromosome 1, play a part in nucleolar organization.

SUMMARY

A new, simple technique for the demonstration of secondary constrictions in human chromosomes is described. A short-term culture of cells in calcium-free medium reveals secondary constrictions at specific loci of certain chromosomes as lightly staining gaps. The general features of secondary constrictions and their position on several chromosomes are described, with some tentative remarks on their identification. Certain problems involving satellite association and nucleolar chromosomes are discussed on the basis of the findings described.

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Tooth Morphology as a Basis for Distinguishing Monozygotic and Dizygotic Twins

ANDERS LUNDSTRÖM

*Department of Orthodontics, Royal School of Dentistry,
Stockholm, Sweden*

THE PRESENT INVESTIGATION is based upon two series of casts, one of 70 twin pairs of like sex collected at the Dental School of the University of Michigan in Ann Arbor, and the other of 54 like sexed pairs at the Dental School of Columbia University in New York. The first series was diagnosed as to zygosity by Sutton, Vandenberg and Clark at the Institute of Human Biology in Ann Arbor and the second by Osborne and De George of the Sloan Kettering Institute of Cancer Research in New York. The casts were made by Dr. J. Alderisio, former Assistant Professor of Orthodontics at the Dental School, University of Michigan, and by Dr. S. Horowitz, Assistant Professor of Orthodontics at the Dental School at Columbia University.

Previous studies

Although there are a number of publications dealing with the genetic control of tooth morphology (Weitz, 1924; Reif, 1928; Korkhaus, 1930, 1939; Kraus, 1951, 1957; Ludwig, 1957) there seems to be only one investigation of the application of genetic variability in tooth form in distinguishing between monozygotic and dizygotic twins (Kraus, Wise and Frei, 1959). Eleven out of 17 earlier described traits for the lower first premolars (Kraus & Furr, 1953) were recorded in four out of six sets of triplets. Concordance and discordance were determined and the frequency of concordance in the possible pairing was used as an indication of zygosity. For all four sets the diagnoses from tooth morphology were identical with the diagnoses on the basis of blood types.

PRESENT INVESTIGATION

Zygosity diagnosis

The zygosity diagnosis of the Ann Arbor material was performed in the following way (Sutton, Vandenberg and Clark, 1962). The twin pairs were first assigned to two groups on the basis of concordance or discordance with respect to blood groups ABO, MN, Rh, Kell and Duffy and the secretor factor. Discordance for one or more of the antisera used (anti-A, absorbed anti-A, anti-B, anti-M, anti-N, anti-C, anti-D, anti-E, anti-c, anti-e, anti-K, and anti-Fy^a) was regarded a sufficient but not a necessary condition for dizygosity. It was estimated that roughly 10 per cent of the concordant twin pairs were dizygous, and several supplementary observations were therefore made to detect

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the dizygous concordant twins, namely, of physical appearance, finger-prints, eye color, visual inspection of head and hand radiographs, and dental casts. A particularly careful examination was made of eye color and iris pattern, which were inspected on Kodachrome transparencies by three observers independently. The concordant twins that all three observers agreed could be distinguished on the basis of the iris patterns were considered to be most probably dizygous.

The use of such a classification for determining the extent to which tooth morphology can be applied in distinguishing monozygous and dizygous pairs might involve some risk of circular reasoning, as an inspection of the dental casts was included in the series of observations conducted for diagnosing dizygous twins. However, the casts obviously played a minor role in the diagnosis and, furthermore, tooth morphology was not referred to specifically in this connection. It is therefore improbable that appreciable bias has been introduced in the present investigation.

Osborne and De George (1959) also based their diagnoses chiefly upon serologic evidence. The blood of the twins was tested for A₁, A₂, B, O; M, N, S; C, D, E, c of the Rh series; Kell, Duffy, and P. A difference in any one of these blood factors was taken as proof that a pair was dizygotic. When, in a supplementary study, five pairs of unlike sex were found to agree as regards all the blood factors tested, it became apparent that it would be necessary to introduce additional diagnostic criteria. For the series studied the following characteristics were found to be of particular value: head hair color, eye color, eye detail pattern, tongue rolling, P.T.C. taste reaction, ear lobe form, chin form, and mid-phalangeal hair. The twin pairs were assigned to three groups: (1) definitely similar, (2) definitely dissimilar or (3) questionably similar in respect of each of these eight characteristics. All twin pairs of like sex for which there was agreement as to blood factors were then separated and classified as *similar*, *dissimilar* or *similar* (?). A twin pair that was recorded as definitely similar for all eight criteria was classed as *similar*. A pair that was recorded as definitely dissimilar in two or more of the eight criteria, or as dissimilar in one and questionable in three or more was classed as *dissimilar*. All others were classed as *similar* (?). Of the 96 pairs of like sex comprising the series, 10 were classified as *dissimilar* and 10 as *similar* (?). The former were listed as dizygotic and the latter as monozygotic. A separate study of dermatoglyphic patterns and facial photographs did not disclose any cause for changing the previous classification. According to Osborne and De George any tendency to error would be toward including closely similar dizygotic pairs in the monozygotic group; for this reason they call their procedure "a proved dizygotic method."

Number of Comparable Tooth Pairs

An important factor in a study of tooth morphology is the number of teeth in the dentition that are available for examination of tooth form. Teeth may be non-available because of non-eruption or extraction, hypodontia, severely defective crowns from caries, enamel hypoplasia, or trauma.

In the present study teeth with, for instance, central or single proximal

fillings were usually found to be acceptable. Where only one tooth of a homologous pair was defective it could still be included in the material. A bilateral comparison is, however, of greater value since it is not uncommon to find small deviations in tooth shape between the left and right sides.

The number of teeth available for co-twin comparison on one or both sides in the Ann Arbor and New York series were on an average 12.4 and 10.3, respectively. This difference is probably due, at least in part, to the fact that the latter series was composed entirely of adult twins (over 18 years) whereas the former included many younger twins with fewer lost or badly decayed teeth. The Ann Arbor series contained only 7 pairs out of 70 having less than 8 teeth comparable against 14 such pairs out of 54 in the New York material.

It is not only the number of available teeth that is of importance but also the types of teeth on which the comparison is based. Teeth with a relatively large variability are of greater value than those with small individual differences. The upper incisors and the lower premolars more often have distinct traits than the lower incisors and canines. The molars and upper premolars seem to assume an intermediate position in this respect. The distribution of the different teeth with respect to their availability to concordance and discordance determinations is shown in tables 1 and 2. In both series the four incisors are best represented (in 93-99 per cent of the Ann Arbor series and in 77-94 per cent of the New York series), whereas the third molars were the least often available (about 3 and 15-17 per cent).

METHOD AND RESULTS

The study was performed by the blind technique. The examiner was unaware of the diagnosis reached on the basis of serologic and general anthropology. The pairs were presented at random so that the observer had no clue as to whether a particular pair of casts belonged to a monozygotic or dizygotic

TABLE 1. INDIVIDUAL TEETH AVAILABLE FOR INTRA-PAIR COMPARISON IN THE ANN ARBOR SERIES (70 TWIN PAIRS)

Intra-pair comparison possible	I ₁	I ₂	C	P ₁	P ₂	M ₁	M ₂	M ₃
Upper Jaw								
Bilaterally	56	58	60	56	41	46	41	—
Unilaterally	9	9	7	12	17	11	15	2
Total	65	67	67	68	58	57	56	2
Percentage of total number of twin pairs (70)	92.9	95.7	95.7	97.1	82.9	81.4	80.0	2.9
Lower Jaw								
Bilaterally	61	59	59	64	46	32	38	1
Unilaterally	5	9	10	5	13	14	14	1
Total	66	68	69	69	59	46	52	2
Percentage of total number of twin pairs (70)	94.3	97.1	98.6	98.6	84.3	65.7	74.3	2.9

twin pair. The casts of the Ann Arbor series were randomized with the help of a table of random numbers. The New York series was collected in such a way that the casts could be expected to be in a random order, and no special measures were taken to ensure randomization. This proved to be not quite satisfactory, since the material contained considerably more monozygotic than dizygotic pairs (34:20), a fact that came to light during the examination and, being unexpected, had a puzzling effect on the examiner. A presentation of the material according to random numbers would probably have been better, although it would then have been necessary to use some dizygotic pairs twice.

To start with, a preliminary examination of the Ann Arbor series was made by three observers. All were orthodontists and had a knowledge of genetics. Observers 1 and 3 had had previous experience of twin investigations on teeth and jaws. Observer 3 (the author) had undertaken a parallel study to the present one on a similar series, for which, however, no serologic examinations had been performed to confirm the diagnoses; he was therefore better trained than observers 1 and 2. The casts of the twin pairs were compared by each observer independently of the other two, and a diagnosis was made on the basis of the shape and, to some extent, the size of the teeth (for differences evident to the unaided eye). The results of these comparisons are shown in table 3.

Observers 1 and 3 both proved to be very reliable in diagnosing the dizygotic pairs (with 3 and 0 of the 32 pairs given as monozygotic) but less reliable in judging the monozygotic pairs (8 and 5, respectively, given as dizygotic). Observer 2 made 8 mistakes for the dizygotic against 2 for the monozygotic. It is conceivable that these discrepancies are manifestations of a systematic difference in judgement, with a tendency for observers 1 and 3 to regard borderline cases as monozygotic and observer 2 to regard them as dizygotic.

Observer 3 afterwards performed a more detailed comparison of the twin

TABLE 2. INDIVIDUAL TEETH AVAILABLE FOR INTRA-PAIR COMPARISON IN THE NEW YORK SERIES (52 TWIN PAIRS FOR THE UPPER AND 53 PAIRS FOR THE LOWER JAWS)

Intra-pair comparison possible	I ₁	I ₂	C	P ₁	P ₂	M ₁	M ₂	M ₃
Upper jaw								
Bilaterally	42	37	38	29	15	25	28	3
Unilaterally	3	10	7	13	15	12	13	5
Total	45	47	45	42	30	37	41	8
Percentage of total number of twin pairs (52)	86.5	90.4	86.5	80.8	57.7	71.2	78.8	15.4
Lower jaw								
Bilaterally	38	40	49	39	21	17	24	4
Unilaterally	3	5	1	11	18	9	9	5
Total	41	45	50	50	39	26	33	9
Percentage of total number of twin pairs (53)	77.3	84.9	94.4	94.4	73.6	49.1	62.3	17.0

casts. Comparisons were made tooth for tooth and the presence of concordance (K), minor (D1) or major discordance (D2) were noted. If there was uncertainty associated with a particular decision, this was indicated by placing a question mark after the notation. When such a record had been made for all the comparable teeth of a twin pair a total evaluation was made and the twin pair in question was denoted as monozygotic (MZ), probably monozygotic (MZ?), probably dizygotic (DZ?) or dizygotic (DZ).

The following types of discordance between homologous teeth were recorded as major:

1. Differences in the number of cusps.
2. Marked differences of fissure-arrangements.
3. Marked form-differences of the crown, *e.g.*, a short and/or broad crown for one tooth and a long and/or narrow for the other.
4. Marked differences in palatal surfaces of upper incisors or canines with, *e.g.*, a rather smooth surface for one tooth and two or more strong enamel ridges for the other.

Drawings were made in order to describe major differences. Minor discordance was recorded for tooth-pairs with less obvious differences, such as more or less curved facial, or lingual surfaces, more round or more angulated occlusal surfaces, more or less pointed or otherwise differently formed cusps.

Examples of the type of differences upon which the tooth-morphology diagnoses were based are given in Fig. 1 and 2.

The results of the classification are given in table 3, line 3b. There is close agreement between the results of the preliminary and detailed examinations. In seven cases the classification was changed: two monozygotic pairs that had first been assigned to DZ? were transferred to MZ; two monozygotic pairs were changed from MZ to MZ?, and one monozygotic pair from MZ? to MZ; one monozygotic pair was changed from DZ to DZ? and one dizygotic pair from DZ? to DZ. Five of the seven changes were thus in the "right" direction and two "wrong." It is not unlikely that in a few cases the recollection of the comparison between the results of the three observers may have had an influence on the detailed evaluation.

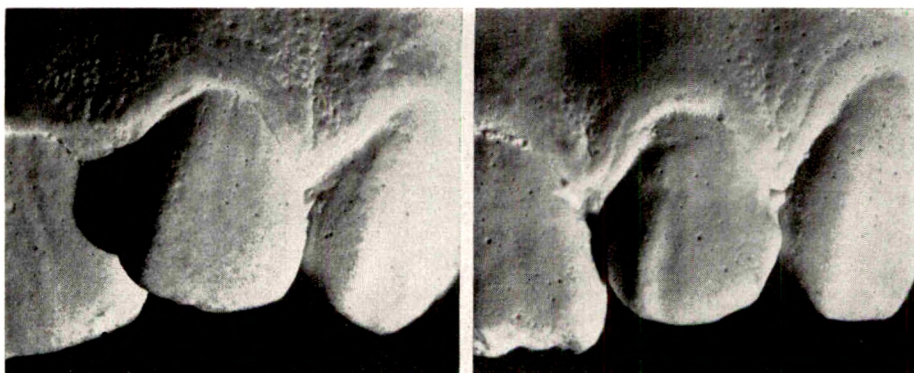


FIG. 1. Upper left lateral incisors from twin pair no. 6, Ann Arbor. According to blood groups and general somatic evaluation this pair was monozygotic. Differences in tooth morphology led to the tooth morphology diagnosis: dizygotic (?).

The diagnosis of the New York pairs was performed by the same detailed procedure as before, but only by observer 3. The results (table 4) are in close agreement with those of the Ann Arbor material. Complete identity was obtained with the original diagnosis for 19 out of 20 pairs that were originally

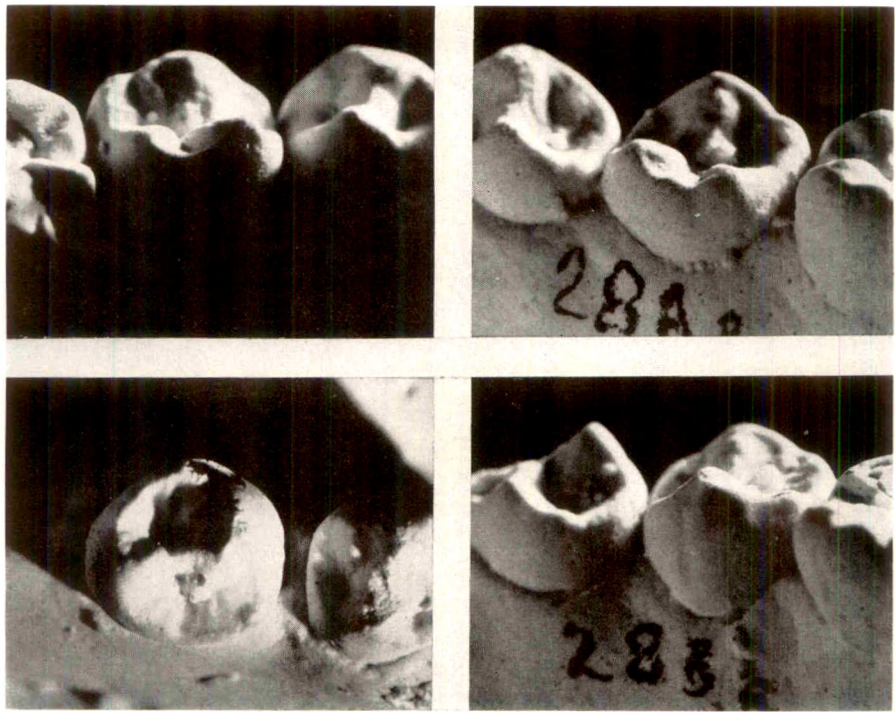


FIG. 2. Lower second premolars from twin pair no. 28, Ann Arbor. According to blood groups and general somatic evaluation this pair was monozygotic. Differences in tooth morphology, especially the fact that the lower second premolars, of one twin had two lingual cusps and those of the other had only one such cusp on both sides, led to the tooth morphology diagnosis: dizygotic.

TABLE 3. CORRESPONDENCE BETWEEN ORIGINAL AND TOOTH MORPHOLOGY DIAGNOSES OF ZYGOSITY, ANN ARBOR SERIES (3 OBSERVERS)

38 monozygous pairs (original diagnosis)					
Tooth-morphology diagnosis		MZ	MZ?	DZ?	DZ
Observer	1	16	14	3	5
	2	33	3	2	0
	3a*	28	5	3	2
	3b	29	6	2	1
32 dizygous pairs (original diagnosis)					
Tooth-morphology diagnosis		DZ	DZ?	MZ?	MZ
Observer	1	27	2	3	0
	2	19	5	5	3
	3a*	31	1	0	0
	3b	32	0	0	0

*For observer 3 the second set of values (3b) reports a more detailed examination.

classified as dizygotic (diagnosis definite for 17 pairs and with reservation for two). The dizygotic pair assessed as monozygotic was recorded as MZ?. This pair was serologically identical, but differed as to iris pattern, PTC tasting, ear lobe and mid-phalangeal hair, and was diagnosed as DZ? by Osborne and De George. Out of the 34 pairs assessed originally as monozygotic, 31 were diagnosed as monozygotic (17 definite and 14 with reservation) and three as dizygotic (two definite and one with reservation). Out of these three pairs one pair was denoted as MZ? by Osborne and De George due to differences in tongue rolling and PTC tasting.

DISCUSSION

A factor of importance in evaluation of the results is the reliability of the original diagnoses. As has already been mentioned, it is easier to establish dizygosity than monozygosity, since evidence for the former is provided by serologic discordance or marked discordance in external features. For this reason, the close agreement obtained in the dizygotic series between the original diagnosis and that made on the basis of tooth morphology would perhaps be expected.

To establish monozygosity is more difficult, however, since a few of the pairs classified as concordant by serologic analysis are in fact dizygotic. By using other anthropologic features it is certainly possible to distinguish most of the pairs of serologically concordant dizygotic twins, but it cannot be ruled out that occasional dizygotic pairs are so closely similar that they cannot be distinguished from monozygotic pairs displaying relatively marked non-genetic difference.

Thus, in cases where dizygosity is indicated on the basis of tooth morphology but monozygosity on the basis of serology and general anthropology, it is not necessarily the latter diagnosis that is the correct one.

It might be mentioned that all six serologically concordant Ann Arbor pairs that were originally designated as DZ were also recorded as DZ on the basis of tooth morphology. For four of these the original diagnosis was DZ and for two DZ?. In the New York series there were also four pairs of the same type (all DZ?). The diagnosis on the basis of tooth morphology was DZ for three of these and MZ? for one.

It is interesting to observe that the proportion of pairs for which the original diagnosis and that based on tooth morphology were not in agreement

TABLE 4. CORRESPONDANCE BETWEEN ORIGINAL AND TOOTH MORPHOLOGY DIAGNOSES OF ZYGOSITY, NEW YORK SERIES

34 monozygous pairs (original diagnosis)					
Tooth-morphology diagnosis		MZ	MZ?	DZ?	DZ
Observer	3	17	14	1	2
20 dizygous pairs					
Tooth-morphology diagnosis		DZ	DZ?	MZ?	MZ
Observer	3	17	2	1	0

was slightly higher for the New York than the Ann Arbor series (7.4 against 4.3 per cent, respectively). The percentage of pairs classed as MZ? and DZ? was also higher for the New York material (33.3 per cent) than for the Ann Arbor material (11.4 per cent). These differences are probably due, at least in some degree, to the greater difficulty of performing a zygoty diagnosis on the basis of tooth morphology in older people. This is also reflected in the number of teeth available for comparison in the two series. Another possible

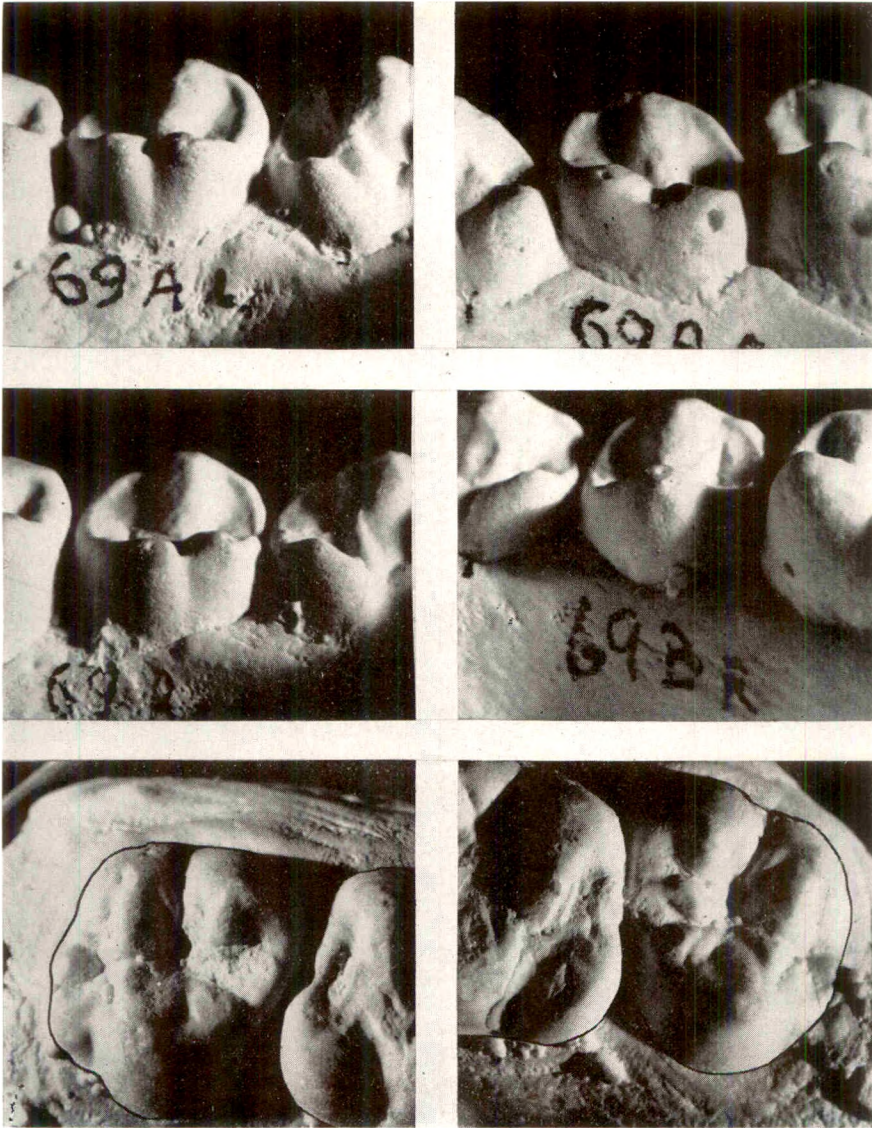


FIG. 3. Lower second premolars from pair no. 69 and upper second molars from one twin of pair no. 65, Ann Arbor. The B-twin of the monozygotic pair no. 69 had two lingual cusps on one side and one lingual cusp on the other side. The A-twin of the dizygotic pair no. 65 shows similar asymmetry of the upper second molars.

explanation of the differences is the unusually large proportion of monozygotic pairs in the New York series. The observer was, as already noted, puzzled thereby and might for this reason have tended to place question marks after his monozygotic diagnoses.

SUMMARY

The results of the study on tooth morphology show that on the whole there was a close agreement with the diagnoses of zygosity made on the basis of general anthropology (including serologic determinations). For 117 out of the 124 pairs studied (94.4 per cent) the same final diagnoses were obtained. The discrepancies demonstrated cannot be proved to be due to incorrect diagnosis on the basis of tooth morphology nor can this possibility be ruled out. In six pairs out of seven the difference consisted of a diagnosis of dizygosity by tooth morphology where monozygosity was diagnosed on grounds of general anthropology (two pairs DZ? and four pairs DZ). In only one pair was a dental morphologic diagnosis of monozygosity (MZ?) made where the anthropologic factors indicated dizygosity (DZ?). This pair was serologically identical, but differed as to iris pattern, PTC tasting, ear lobe and mid-phalangeal hair.

The presence of a difference in the number of cusps on the left and right sides, whereby, for instance, the lower second premolar had one lingual cusp on one side and two such cusps on the other (Fig. 3), indicates a variation in the expressivity of the genes, even in the case of a character determined so strongly by heredity as tooth morphology. This means that the different shape of a particular tooth of two twins of a pair cannot be regarded as proof of dizygosity, even if the discrepancies are bilateral (Fig. 2). If other teeth are strikingly similar in form, monozygosity is probably the correct diagnosis. If the present study had been based on this principle two Ann Arbor pairs would probably have been diagnosed as monozygotic, as was indicated by the general anthropology.

As a general conclusion it would seem that for twin pairs for which a large enough number of permanent teeth are available for comparison of tooth morphology, a fairly reliable judgment can be made on whether there is monozygosity or dizygosity by an observer having a good knowledge of the variation in tooth morphology.

ACKNOWLEDGMENTS

I would like to take this opportunity of expressing my appreciation of the kindness of Professor R. E. Moyers and Professor N. Di Salvo, heads of the Orthodontic Departments at the Dental Schools of the University of Michigan, Ann Arbor, and Columbia University, New York, for placing the facilities of their Departments at my disposal for this investigation. I am also grateful to Dr. R. H. Osborne of the Sloan Kettering Institute of Cancer Research for providing valuable data on the New York series of twins, and to Drs. S. Hunter, J. Harris and S. Horowitz for their collaboration.

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The Distribution of the Gamma Globulin Types Gm(a), Gm(b), Gm(x) and Gm-like in South and Southeast Asia and Australia

G. H. VOS¹, R. L. KIRK² AND A. G. STEINBERG³

WITH THE TECHNICAL ASSISTANCE OF DELL VOS AND RACHEL STAUFFER

SEVERAL STUDIES OF THE distribution of the Gm phenotypes in populations from various parts of the world have been reported. The results of these studies have recently been summarized by Steinberg (1962) and a comprehensive set of tables is in course of preparation by Ropartz, Rousseau and Rivat (1962b). In broad outline it seems that Caucasian populations possess the alleles Gm^a , Gm^{ax} , and Gm^b , that Mongoloid populations possess the alleles Gm^a , Gm^{ax} and Gm^{ab} , whilst Negroes possess only Gm^{ab} . The Gm-like factor is present in Negroes but is completely absent in whites. Steinberg *et al.* (1961b) found 43.6 per cent of 149 Micronesians from Rongelap Atoll to be Gm-like (+) as well as 62.9 and 50.0 per cent, respectively, of a small series of Djuka Negroes and Javanese from Surinam. Ropartz *et al.* (1962c) have also reported 4 per cent Gm-like (+) individuals among a small sample of Chinese from Macao.

During the last two years we have had the opportunity to study serum samples collected in various parts of south and southeast Asia and in Australia. These samples have come from different ethnic groups, including tribal and modern populations. It is the purpose of the present paper to record the Gm types and gene frequencies for these populations.

MATERIALS AND METHODS

1. Australia: The aboriginal populations comprise two separate groups, one from the Western Desert, stretching from Leonora in Western Australia eastwards to the Rawlinson Ranges, the other made up of several different tribes in process of disintegration in the Kimberley area of Western Australia, and sampled in the Derby and Halls Creek townships. The white population consisted of non-selected blood samples collected from donors of the Western Australia Red Cross Blood Transfusion Service.

2. Malaya: Two separate series of Indians and Chinese have been studied. In the first series samples were collected in Kuala Lumpur and Perlis. The Indians in this series were a mixed group of Tamils and Sikhs. The second series consisted of the parents of the families recorded in the study of Steinberg *et al.* (1961a). The Malay samples were collected almost entirely in Perlis.

3. Thailand: The samples from Thais were obtained from blood donors in Bangkok. Care was taken to exclude persons with known recent Chinese ancestry.

4. India: Several tribal populations were studied. The Oraons were sampled in the

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¹Pathology Laboratory, King Edward Memorial Hospital, Subiaco, Western Australia.

²Zoology Department, The University, Nedlands, Western Australia.

³Biology Laboratory, Western Reserve University, Cleveland, Ohio.

villages of Barahai Rol, Bijupara and Sosai Ashram near Ranchi on the Chota Nagpur plateau. The Todas, Kurumbas and Irulas were sampled in villages near Coonoor and Ootacamund, in the Nilgiri Hills, South India.

5. Ceylon: Sinhalese blood samples were collected mainly from places in or near Colombo and the Tamils from Kurunegara in North Central Province. The Veddah samples were obtained from the villages of Ginindamana and Dalukana and the Wannu castes were obtained from villages in the North Central Province.

6. Pakistan: Punjabi samples came from two sources in Lahore, workers and students in two hospitals and patients of a large mental institution. The Pathans were from Peshawar and surrounding areas, and the persons sampled were screened carefully for non-Pathan ancestry.

The samples from the modern populations were from non-related persons. In the case of the tribal populations, however, it was not always possible to rule out some degree of relationship between persons in the series. Some of the populations are very small, the Todas in South India and Veddahs in Ceylon, for instance, numbering approximately only 700 and 400, respectively. This lack of randomness applies also to the small caste populations of the Wannu in Ceylon. No correction has been made for the presence of related individuals in the calculation of the gene frequencies, but it is considered that the values obtained are a useful approximation for the populations being studied.

Blood was drawn into Bayer "Venules" or Becton-Dickinson "Vacutainers", placed into ice-cold containers and flown to Perth with minimum delay. Serum separated from the clots was stored at -20°C . until ready for typing. Aliquot portions of the samples in some of the series were flown to Cleveland in dry-ice for duplicate testing. Where discrepancies occurred in the typing results of the two laboratories the tests were repeated independently until agreement was obtained.

Table 1 lists the anti-Rh sera, agglutination sera and the dilutions of the test sera employed during the present investigation. The methods of testing were essentially identical with those outlined by Steinberg *et al.* (1961b).

RESULTS AND DISCUSSION

Table 2 records the phenotype frequencies of the Gm (a), Gm (b) and Gm (x) factors, together with gene frequencies for the alleles Gm^a , Gm^{ax} , Gm^b and Gm^{ab} . The gene frequencies were obtained by using the maximum likelihood equations derived by Steinberg *et al.* (1961b), with the exception of the Oraons. This population required the presence of four alleles to satisfy equilibrium conditions and a fresh set of equations had to be derived to calcu-

TABLE 1. REAGENTS USED FOR GM TYPING

Original Typing					
Gm type	Rh anti-sera		Agglutinator	Dilution of Agglutinator	Dilution of Test Serum
Gm (a)	Kim	1/10	Scol	1/8	1/8, 1/16
	Kim	1/10	Wils	1/8	1/8, 1/16
	Mann	1/10	Cipora	1/8	1/8, 1/16
	Hasting	1/10	Higham	1/8	1/8, 1/16
Gm (b)	Ham	1/10	Bomb	1/32	1/8, 1/16
	Pac	1/10	Bomb	1/32	1/8, 1/16
Gm (x)	Ham	1/10	Bowers	1/64	1/16, 1/32
Gm-like	Warren	1/10	Bomb	1/32	1/16, 1/32
	Warren	1/5	Carp	1/32	1/16, 1/32
Confirmatory Typing					
Gm (a)	251	1/10	Wils	1/16	1/8, 1/16
	Ham	1/10	Rh 7	1/16	1/8, 1/16
Gm (b)	Berg, A.	1/5	Berg, N.	1/30	1/8, 1/16
Gm (x)	MAG	1/10	Bowers	1/64	1/4, 1/8
Gm-like	Warren	1/10	PCE 64	1/32	1/8, 1/16

TABLE 2. GM PHENOTYPES AND GENE FREQUENCIES FOR VARIOUS POPULATIONS IN SOUTH AND SOUTH EAST ASIA AND AUSTRALIA

POPULATION	No. Tested	Phenotype Frequencies				Gene Frequencies				χ^2	D.F.	P						
		Gm(a+b-x-) No.	%	Gm(a+b-x+) No.	%	Gm(a+b+x-) No.	%	Gm ^a	Gm ^b				Gm ^a b					
Australia																		
Whites	300	20	6.8	22	7.3	91	30.3	37	12.3	130	43.3	0.251	0.103	0.646	—	1.484	2	.50>P>.30
Aborigines Western Desert	289	154	53.3	135	46.7	—	—	—	—	—	—	0.730	0.270	—	—	—	—	—
Aborigines Kimberleys	268	83	31.0	103	38.4	65	24.3	17	6.3	—	—	0.577	0.256	—	0.167	2.885	1	.10>P>.05
Ceylon																		
Sinhalese	159	43	27.0	47	29.6	47	29.6	11	6.9	11	6.9	0.545	0.203	0.252	—	3.028	2	.30>P>.20
Tamils	108	39	36.1	23	21.3	33	29.6	9	8.3	5	4.6	0.603	0.161	0.236	—	0.320	2	.90>P>.80
Wanni Castes	98	10	10.2	19	19.4	49	50.0	9	9.2	11	11.2	0.437	0.155	0.408	—	12.997	2	.01>P>.001
Veddahs	52	6	11.5	3	5.8	23	44.2	1	2.0	19	36.5	0.364	0.040	0.596	—	2.269	2	.50>P>.30
India																		
Oraons	124	4	3.2	1	0.8	105	84.7	3	2.4	11	8.9	0.182	0.018	0.297	0.503	0.117	1	.80>P>.70
Todas	99	4	4.0	3	3.0	30	30.0	5	5.0	57	58.0	0.204	0.041	0.755	—	1.022	2	.70>P>.50
Kurumbas	52	7	13.5	3	5.8	24	46.1	4	7.7	14	26.9	0.392	0.070	0.538	—	0.399	2	.90>P>.80
Iruilas	74	14	18.9	4	5.4	22	29.7	12	16.3	22	29.7	0.358	0.115	0.527	—	5.828	2	.10>P>.05
Malaya																		
Indians (mixed)	60	16	26.7	11	18.3	26	43.3	6	10.0	1	1.7	0.563	0.153	0.284	—	6.151	2	.05>P>.02
Indians (parents)	128	39	30.5	36	28.1	30	23.4	17	13.3	6	4.7	0.535	0.234	0.231	—	1.336	2	.70>P>.50*
Chinese 149*	7	4.7	4	2.7	122	81.9	14	9.4	2	1.3	0.233	0.063	—	0.705	0.130	1	.80>P>.70	
Chinese (parents)	90	4	4.4	2	2.2	81	90.0	3	3.3	—	—	0.231	0.028	—	0.741	0.686	1	.50>P>.30*
Malays 156*	4	2.6	3	1.9	132	84.6	16	10.2	1	0.6	0.166	0.063	—	0.772	0.241	1	.70>P>.50	
Pakistan																		
Punjabis	203	24	12.0	16	8.0	87	42.4	15	7.5	61	30.1	0.369	0.081	0.550	—	1.766	2	.50>P>.30
Pathans	109	12	11.0	8	7.3	40	36.7	11	10.1	38	34.9	0.326	0.091	0.583	—	0.181	2	.95>P>.90
Thailand																		
Thais	163	8	4.9	6	3.7	132	81.0	17	10.4	—	—	0.220	0.073	—	0.707	0.014	1	.95>P>.90

*The Gm (a-) phenotypes were ignored in calculating the gene frequencies.

* χ^2 and P from Steinberg *et al.*, 1961a.

late the gene frequencies for this situation. The equations are given in appendix 1.

Goodness of fit assuming Hardy-Weinberg equilibrium was estimated in the usual way, and the χ^2 values are included in table 1. The only population which reveals a significant departure from Hardy-Weinberg equilibrium is that of the Wannni castes in Ceylon. Here there is a significant excess of the Gm ($a+b+x-$) phenotype, but it seems unlikely that this is due to the presence of a Gm^{ab} allele in this population. Historically the Wannni castes are said to be derived from Sinhalese with Tamil admixture, and a careful study of other blood group and serum group systems supports this view (Kirk *et al.*, 1962b). It seems more likely that the discrepancy is due to lack of randomness, particularly in the samples from the Badal and Berava castes which make up about 60 per cent of the total number in the Wannni series.

The main features of the Gm gene distribution for the whole area are shown diagrammatically on the map of Fig. 1. In south and southeast Asia two types of populations may be distinguished. The Mongoloid Chinese, Malay and Thai populations are characterized by relatively high frequencies of the allele Gm^{ab} , moderate frequencies of Gm^a and low frequencies of Gm^{ax} . In contrast the populations of India and Ceylon, with the exception of the Oraons of the Chota

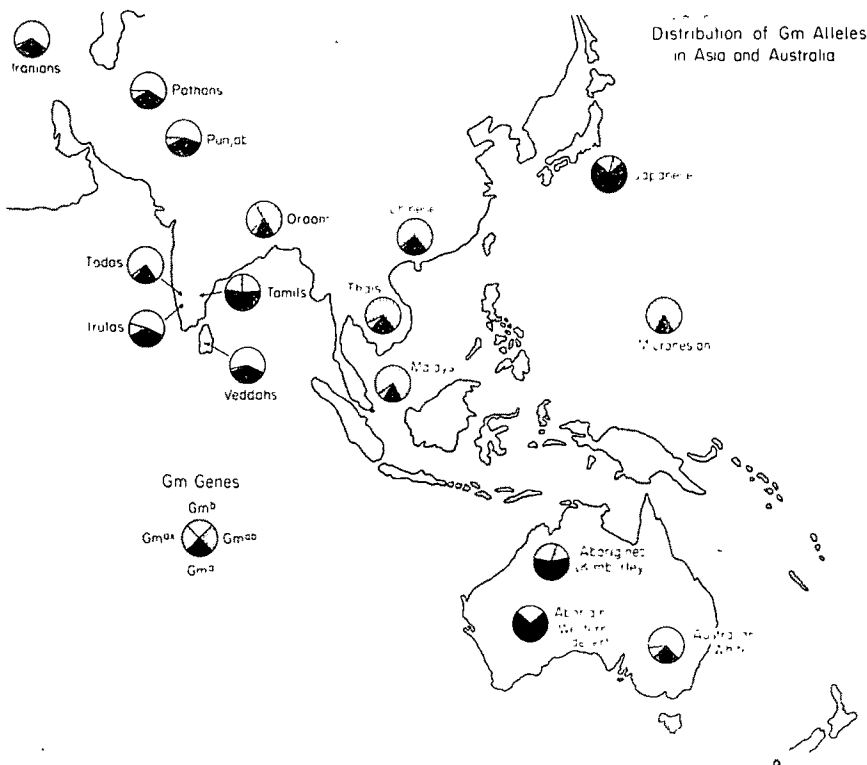


FIG. 1. Map showing the distribution of Gm alleles in various populations in South and Southeast Asia and Australia. The Chinese and Tamils were sampled in Malaya but are shown on the map in the area of their origin. The Iranians, Japanese and Micronesians have been included for purposes of comparison.

Nagpur Plateau, have the three alleles Gm^a , Gm^{ax} and Gm^b , but no Gm^{ab} . The Gm^{ax} frequency is low except among the Tamils of South India, where it achieves a frequency of 23 per cent. The Gm^a and Gm^b alleles fluctuate over a wide range of values, Gm^a reaching its highest value of 60.4 per cent among the Tamils of Ceylon.

In northwest India and Pakistan lower values of Gm^a with correspondingly higher values of Gm^b are found among the Punjabis and Pathans, suggesting a clinal decrease in the Gm^a frequency toward the lower values characteristic of Caucasian populations in Europe. In this respect the frequencies of the Gm alleles among the Todas of south India are of particular interest. The low Gm^a and high Gm^b frequencies of the Todas distinguishes them from the neighbouring veddoid Irula, and Kurumba tribes in the Nilgiri hills, and also from the south Indian Tamil populations. The values among the Todas are more similar to a south European or Middle-east population. The Gm frequencies of the Iranians (Ropartz *et al.* 1962a) are not significantly different from those of the Todas. Kirk and Lai (1961) have shown that the haptoglobin frequencies of the Todas are also compatible with the hypothesis that they come from a population to the north and west of their present territory.

The Oraons of the Chota Nagpur Plateau, in northeast India pose another interesting problem for the anthropologist. To satisfy Hardy-Weinberg equilibrium it is necessary to assume the presence of the allele Gm^{ab} as well as the alleles Gm^a , Gm^{ax} and Gm^b . This is a reasonable assumption if one supposes that the Oraons have had genes from neighboring Mongoloid populations introduced into their population. Kirk *et al.* (1962a) have shown that several characteristics of the Oraons suggest a strong Mongoloid influence in the genetic pool of the Oraons. The Rh gene frequencies are more similar to those of southeast Asian populations than to modern Indian populations. Further, the Oraons are the only people studied so far in India who possess the Diego factor Di (a) (Vos and Kirk, 1961), and also transferrin D, both characteristic of Mongoloid populations.

It is interesting to contrast the relatively uniform frequencies of Gm^a , Gm^{ax} and Gm^{ab} among the Chinese, Thais and Malays, with the entirely different values reported for the Japanese (Ropartz *et al.* 1961; Steinberg *et al.* 1961a). Among the Japanese the frequency of the Gm^{ab} allele has been reduced to approximately 10 per cent, and Gm^b appears to be absent. Although the Japanese differ genetically to some extent from other populations in eastern Asia, particularly with respect to the frequency of blood group gene *M* and the Rh chromosome *R*², the discrepancies with respect to the Gm alleles are so striking that they suggest that some other phenomenon is present which is responsible for the widely differing Gm frequencies.

In Australia one series of Caucasians and two aboriginal populations have been studied. The Caucasian series shows the alleles Gm^a , Gm^{ax} and Gm^b to be present with frequencies similar to those characteristic of north European populations (Steinberg, 1962). The two aboriginal populations, however, have quite different frequencies for the Gm alleles. Aborigines from the Western Desert possess only the alleles Gm^a and Gm^{ax} . The absence of both Gm^b and Gm^{ab} is not a highly localized phenomenon, for the Western Desert group is

made up of samples collected from Leonora, at the western extreme of the range, Cundeelee in the south to the Rawlinson Ranges 600 miles northeast. Out of nearly 300 sera from this area that have been Gm typed, only three gave Gm (b+) results. Each of these three individuals was found to have some white ancestry, and has been excluded from the present series.

The aborigines from the Kimberley area show a high frequency of the Gm^{ax} allele. The allele Gm^b is not present, but Gm^{ab} reaches a value of 16.7 per cent. It is interesting to note here that these same two aboriginal populations from the Western Desert and the Kimberleys do not differ significantly in the frequency of the Inv (a+) phenotype (Ropartz *et al.* 1962d). Further study of sera from aborigines in other parts of the Australian continent and from tribal populations in New Guinea will be necessary to delineate more clearly the pattern of the Gm gene distribution in this part of the world.

Steinberg *et al.* (1961b) have shown already that the Gm^{ab} allele is present in high frequency among Micronesians. Together with its presence in Australian aborigines, and among Mongoloid populations, it appears that this allele has a wide distribution elsewhere in the world outside Africa, where it achieves a frequency of 100 per cent in Negro populations.

It is possible that the development of new test systems may reveal that the Gm^{ab} allele of Mongoloid populations, or among the Australian aborigines, is not identical with that of Africans. Already we have some preliminary evidence that this is the case, but further study is needed to clarify this situation.

Tests for Gm-like were carried out for some of the populations reported on above. No Gm-like (+) persons were found among the two series of Australian aborigines, the Oraons, and the Indian and Chinese parent series which formed part of the previous studies by Steinberg *et al.* (1961a). Among the Thais one person was recorded as Gm-like (+), and two others after careful titration of the sera were scored as intermediate for the Gm-like reaction. Despite these results, however, it seems that the phenotype Gm-like is absent throughout the whole area of the present investigation, at any rate with the intensity with which it can be demonstrated in Gm-like (+) individuals among Africans.

SUMMARY

Over 2,600 sera from many parts of south and southeast Asia and Australia have been typed for the factors Gm (a), Gm (b) and Gm (x). Many of these sera have been typed also for Gm-like.

Mongoloid populations have the three alleles Gm^a , Gm^{ax} and Gm^{ab} . Both modern and tribal populations in Ceylon, India and Pakistan, on the other hand, have the three alleles Gm^a , Gm^{ax} and Gm^b , with the exception of the Oraons. These people have, in addition to the three alleles present in other Indian populations, the allele Gm^{ab} characteristic of Mongoloid populations to the East.

Two aboriginal populations in Australia show significant differences. In the Western Desert group Gm (b) is completely absent, whilst in the Kimberley area of Western Australia Gm^a , Gm^{ax} and Gm^{ab} are present, but the Gm^{ab} allele has the low frequency of 16.7 per cent.

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APPENDIX

Derivation of maximum likelihood equations for estimating the frequencies of the alleles Gm^a , Gm^{ax} , and Gm^{ab} , and Gm^b in the Oraons.

Let the frequency of $Gm^a = p$

$$Gm^{ax} = q$$

$$Gm^{ab} = r$$

$$Gm^b = s,$$

$$\text{and let } p + q = u$$

$$r + s = v.$$

Note that $p + q + r + s = u + v = 1$.

OBSERVED PHENOTYPES

(only positive reactions are shown)

Number observed	$\frac{a}{C}$	$\frac{ax}{D}$	$\frac{ab}{E}$	$\frac{axb}{F}$	$\frac{b}{G}$	$\frac{\text{Total}}{T}$
Expected frequency	p^2	$q^2 + 2pq$	$r^2 + 2pr + 2ps + 2rs$	$2qs + 2qr$	s^2	1

Expected frequency of $Gm(a) + Gm(ax) =$

$$p^2 + 2pq + q^2 = (p + q)^2 = u^2.$$

Expected frequency of $Gm(ab) + Gm(axb) + Gm(b) =$

$$\begin{aligned} & r^2 + 2pr + 2ps + 2rs + 2qs + 2qr + s^2 \\ &= (r^2 + 2rs + s^2) + 2p(r + s) + 2q(r + s) \\ &= (r + s)^2 + 2(r + s)(p + q) = v^2 + 2uv \\ &= (1 - u)^2 + 2u(1 - u). \end{aligned}$$

The likelihood expression is:

$$L = \ln K + (C + D) \ln(u^2) + (E + F + G) \ln[(1 - u)^2 + 2u(1 - u)].$$

$$= \ln K + 2(C + D) \ln u + (E + F + G) \ln(1 - u^2), \text{ where } K = \text{a constant.}$$

Differentiating with respect to u and setting the differential equal to zero we have,

$$\frac{dL}{du} = \frac{2(C + D)}{u} - \frac{2(E + F + G)u}{1 - u^2} = 0.$$

$$\text{Hence } u^2 = \frac{C + D}{T},$$

$$u = \sqrt{\frac{C + D}{T}} = p + q, \quad (1)$$

$$\text{and } v = 1 - \sqrt{\frac{C + D}{T}} = r + s. \quad (2)$$

If we estimate p or q , and r or s , we will have estimates of all four alleles.

The likelihood expression for the five phenotypes is (letting $q = 1 - p - r - s$):

$$\begin{aligned} L &= \ln K' + 2C \ln p + D \ln [(1 - p - r - s)^2 + 2p(1 - p - r - s)] \\ &+ E \ln (r^2 + 2pr + 2ps + 2rs) + F \ln 2(1 - p - r - s)(s + r) + 2G \ln s. \\ &= \ln K' + 2C \ln p + D \ln (1 - p - r - s) + D \ln (1 + p - r - s) \\ &+ E \ln (r^2 + 2pr + 2ps + 2rs) + F \ln 2 + F \ln (1 - p - r - s) + F \ln (r + s) + G \ln s. \end{aligned}$$

The partial differentials (equated to zero) with respect to p , r , and s are:

$$\begin{aligned} \frac{\partial L}{\partial p} &= \frac{2C}{p} - \frac{D}{1 - p - r - s} + \frac{D}{1 + p - r - s} + \frac{E(2r + 2s)}{r^2 + 2pr + 2ps + 2rs} - \\ &= \frac{2C}{p} - \frac{D}{1 - p - r - s} + \frac{D}{1 + p - r - s} + \frac{2E(r + s)}{r^2 + 2rs + 2p(r + s)} - \end{aligned}$$

$$= \frac{2C}{p} - \frac{D}{1-p-v} + \frac{D}{1+p-v} + \frac{2Ev}{v^2-s^2+2pv} - \frac{F}{1-p-v} = 0 \quad (3)$$

$$\begin{aligned} \frac{\delta L}{\delta r} &= \frac{-D}{1-p-r-s} - \frac{D}{1+p-r-s} + \frac{E(2r+2p+2s)}{r^2+2pr+2ps+2rs} - \\ &\quad \frac{\frac{F}{1-p-r-s}}{\frac{F}{1-p-r-s} + \frac{F}{r+s}} \\ &= \frac{-D}{1-p-v} - \frac{D}{1+p-v} + \frac{2E(v+p)}{v^2-s^2+2pv} - \frac{F}{1-p-v} + \frac{F}{v} = 0 \end{aligned} \quad (4)$$

$$\begin{aligned} \frac{\delta L}{\delta s} &= \frac{-D}{1-p-r-s} - \frac{D}{1+p-r-s} + \frac{E(2p+2r)}{r^2+2pr+2ps+2rs} - \\ &\quad \frac{\frac{F}{1-p-r-s} + \frac{2G}{r+s}}{\frac{F}{1-p-r-s} + \frac{F}{r+s} + \frac{2G}{s}} \\ &= \frac{-D}{1-p-v} - \frac{D}{1+p-v} + \frac{2E(p+r)}{v^2-s^2+2pv} - \frac{F}{1-p-v} + \frac{F}{v} + \\ &\quad \frac{2G}{s} = 0 \end{aligned} \quad (5)$$

The simultaneous solution of equations (3), (4), and (5) would yield maximum likelihood values for p , r and s , but we have been unable to obtain these solutions. Accordingly, we have solved them as follows and have used the values of u and v derived above for the determination of p , r , and s .

$$(3) - (4) = \frac{2C}{p} + \frac{2D}{1+p-v} - \frac{2Ep}{v^2-s^2+2pv} - \frac{F}{v} = 0$$

and

$$(4) - (5) = \frac{2Ev - 2Er}{v^2-s^2+2pv} - \frac{2G}{s} = 0.$$

Since $v - r = s$,

$$(4) - (5) = \frac{Es}{v^2-s^2+2pv} - \frac{G}{s} = 0,$$

$$\text{and } s^2 = \frac{Gv(v+2p)}{E+G}. \quad (6)$$

$$s = \sqrt{\frac{Gv(v+2p)}{E+G}}. \quad (6a)$$

Substituting for s^2 in (3) - (4) we have

$$\begin{aligned} &\frac{2C}{p} + \frac{2D}{1+p-v} - \frac{2Ep}{v^2 - \frac{Gv(v+2p)}{E+G} + 2pv} - \frac{F}{v} \\ &= \frac{2C}{p} + \frac{2D}{1+p-v} - \frac{2p(E+G)}{v(v+2p)} - \frac{F}{v} = 0. \end{aligned} \quad (7)$$

Equation (7) can be solved for p by iteration, using the square root of the frequency of the G_m ($a + x - b -$) class as the initial trial value and the value of v (.8) derived from equation (2). In this way we found $p = .182$. Substitution of this value of p in equation (6a) provides the value of s (.297). Since $u = p + q$ and $v = r + s$ and u , v , p , and s are known, q and r may be obtained.

Genetic Considerations in Familial Hemorrhagic Disease

I. The Sex-Linked Recessive Disorders, Hemophilia and PTC Deficiency

JESSICA H. LEWIS, PAUL DIDISHEIM,¹ JOHN H. FERGUSON
AND C. C. LI

*Department of Medicine, School of Medicine, and Department of Biostatistics,
Graduate School of Public Health, University of Pittsburgh;
Department of Physiology, University of North Carolina*

THE STARTLING AND OFTEN fatal occurrence of hemorrhage in various members of the same family led to early recognition of the fact that hemorrhagic disease is often an inherited characteristic. The first description in the medical literature, by Dr. John Otto in 1803, concerned a severe hemorrhagic disorder occurring in males but transmitted by females. Today we recognize many different types of hemorrhagic disease due to deficiencies of coagulation factors, of platelet abnormalities, or to defects in the vascular system. This communication concerns two of the most common: Hemophilia (AHF deficiency) and PTC deficiency (Hemophilia B or Christmas disease). Although these disorders are due to deficiencies of distinctly different proteins, they are similar in clinical symptoms and in inheritance pattern. Both occur almost entirely in males, and are due to a partially recessive gene on the X chromosome (Didisheim, Ferguson and Lewis, 1958; Graham, Collins, Godwin and Brinkhous, 1953; Verstraete and Vandenbroucke, 1955; Didisheim and Vandervoort, 1962).

Clinical Material: This study concerns 202 patients suffering from and 136 carriers of hemophilia and 60 patients and 35 carriers of PTC deficiency. These individuals have been seen over a ten-year period and come from two different geographical areas: North Carolina and Western Pennsylvania. Slight differences in racial background were noted. In North Carolina two AHF and three PTC and in Pennsylvania two AHF and one PTC kindreds were Negro. The North Carolina families were long settled "old American," predominantly English, while the Pennsylvania families were primarily "second generation American," predominantly middle European.

The 150 hemophiliacs (AHF) seen in Pittsburgh were derived from an

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¹Recipient of Post-doctorate Fellowship, Life Insurance Medical Research Fund.

Present address: Department of Medicine, College of Medicine, University of Utah, Salt Lake City, Utah.

estimated population of 2 million, suggesting that the occurrence of this disease is at least one in 10,000 in the United States.

Clinical Description: Hemophilia and PTC deficiency have entirely similar manifestations. In both the bleeding tendency varies among individuals from very mild to very severe, but in a given individual and in affected members of his family the severity of the affliction remains fairly constant throughout life. The most common symptoms are easy bruising, bleeding following injury, tooth extraction or operation, hemarthrosis, hematuria, and epistaxis. We have arbitrarily divided the patients into four groups depending upon the severity of bleeding judged by history (Lewis, Ferguson, Fresh and Zucker, 1957). Mild (+): posttraumatic bleeding only, e.g., easy bruising, excessive bleeding from cuts, scratches, tooth-extractions, tonsillectomy, other surgery, and epistaxis (often noted in childhood). Moderate (+ +): besides the above, an occasional major bleeding episode such as hemarthrosis, hematuria, hematemesis, melena, hemoptysis, internal bleeding, etc. Severe (+ + +): two to five major episodes per annum. Very severe (+ + + +): six or more major episodes a year.

The carriers of hemophilia or PTC deficiency studied consisted of the following groups: (1) daughters of a hemophiliac, (2) mothers of two or more hemophiliacs or carriers (3) mothers of a single hemophiliac in a family with a clear-cut history of bleeding in other male relatives, and (4) mothers of only one hemophiliac in a family devoid of other bleeding history. It is possible that the latter (group 4) are not true carriers and that the genetic change occurred in their gametes or in the hemophiliac himself. Carriers are usually asymptomatic. Occasionally a history of mild bleeding (\pm) is elicited but this is not unlike that described by many normal women. Rarely a definite (+) hemorrhagic history is obtained. The most common symptoms are easy bruising and post-operative hemorrhage.

Laboratory Tests: AHF and PTC were assayed by methods previously described (Lewis *et al.*, 1957 a, b). Plasma levels of AHF and PTC in normal subjects lie between 50 and 150 per cent, the distribution following the usual bell-shaped curve. Extensive coagulation tests were carried out on all patients and all carriers at times during which they were not under treatment. In patients the critical diagnostic observation was a low plasma level of AHF or PTC. These two coagulation factors are easy to distinguish by laboratory test. In our series a patient deficient in one was always normal in the other. The only other coagulation abnormalities found in these two groups of patients were in the non-specific tests such as blood clotting time, prothrombin consumption and thromboplastin generation. No patient with a consistently prolonged bleeding time has been included, thus eliminating the possibility of confusion with patients suffering from von Willebrand's disease. In addition, no patient with an "acquired" AHF or PTC deficiency was included. Acquired deficiencies of AHF are very rare and usually associated with circulating inhibitors. Acquired deficiencies of PTC are quite common, occurring in conjunction with liver disease, vitamin K deficiency and dicumarol therapy.

Relationship between AHF or PTC Level and Clinical Symptoms: Table 1 illustrates the fairly close correlation between AHF or PTC level and hemorrhagic symptoms. As shown in the last column there are more patients with

extremely low levels of AHF than of PTC, but it should be emphasized that in either deficiency the plasma levels may be zero or as high as 20 per cent of normal. Many patients have been tested repeatedly over a seven-year period, without detection of fluctuating levels.

Plasma Levels of AHF or PTC and Hemorrhagic Symptoms in Carriers: Table 2 shows the plasma level of AHF in hemophilia carriers and of PTC in PTC deficiency carriers. For convenience carriers are placed in three groups, those with definitely abnormal levels (45 per cent or less), those with borderline levels (50 to 60 per cent) and those with normal levels (65 per cent or greater). Sixteen per cent of the carriers of AHF and 37 per cent of the carriers of PTC were abnormal (45 per cent or less) by laboratory test. Relatively few of these gave a history of significant bleeding tendency. Many carriers have been tested repeatedly and, again, only minor fluctuations in AHF or PTC level found.

Kindreds: The 202 AHF deficient patients studied came from 120 different families; the 60 PTC deficient patients from 31 families. The kindreds (table 3) were divided into 3 types: type 0, containing only one known bleeder; type 1, containing two or more bleeders in a single sibship; type 2, containing two or more bleeders in different sibships. Approximately one-third of all families contained only one bleeder.

Relationship between Type of Family History and Severity of Coagulation Defect: Table 4 shows the distribution of studied patients and carriers in the three types of kindred, subdivided as to their AHF or PTC levels. Sixty (77 per cent) of the 78 hemophilia patients coming from families containing affected individuals in only one sibship (types 0 and 1) showed AHF levels in the lowest range, while only 51 (41 per cent) of 124 patients from type 2

TABLE 1. RELATIONSHIP BETWEEN SYMPTOMS AND COAGULATION DEFECT

	Plasma Level (%)	Hemorrhagic Symptoms					Total	
		++	3+	2+	1+	*	No. Patients	%
AHF—202 patients	0 — 0.3	72	34	2	0	3	111	55
	0.4— 1.0	3	21	9	1	0	34	17
	1.1— 5.0	0	2	18	8	1	29	14
	6.0—20.0	0	1	9	18	0	28	14
PTC—60 patients	0 — 0.3	12	6	3	0	0	21	35
	0.4— 1.0	0	5	3	2	0	10	17
	1.1— 5.0	0	1	11	2	1	15	25
	6.0—20.0	0	0	4	10	0	14	23

*Infants not graded as to clinical severity.

TABLE 2. RELATIONSHIP BETWEEN SYMPTOMS AND COAGULATION DEFECT IN CARRIERS

	Plasma Level (%)	Hemorrhagic Symptoms			Total No. Patients	%
		+	±	0		
AHF—136 carriers	45 or <	3	10	9	22	16.1
	50-60	0	8	29	37	27.2
	65 or >	0	6	71	77	56.6
PTC—35 carriers	45 or <	2	3	8	13	37.1
	50-60	0	4	5	9	25.7
	65 or >	0	4	9	13	37.1

families had such extreme deficiencies. Comparable figures for PTC deficiency were 9 (56 per cent) of 16 patients from type 0 and 1 kindreds and 12 (27 per cent) of 44 patients from type 2 kindreds. Carriers with detectable coagulation defects (45 per cent or less) occur slightly more frequently in types 0 and 1 than in type 2 kindreds, *i.e.*, 10 (21 per cent) of 47 AHF and 7 (54 per cent) of 13 PTC females from families in whom their sons were the only known bleeders showed abnormal plasma factor levels, whereas from type 2 kindreds, only 12 (13 per cent) of 89 and 6 (27 per cent) of 22 were equally low.

Relationship between AHF or PTC Level in a Carrier and in Her Son. The data presented in table 5 suggest that the level of AHF or PTC in a carrier is more likely to be abnormally low if her son's deficiency is severe (0 — 0.3 per cent) than if it is milder. Thus, 21.2 per cent of these AHF mothers and

TABLE 3. DISTRIBUTION OF PATIENTS AND KINDREDS AS TO TYPE

	No. and % of Bleeders				No. and % of Kindreds			
	AHF		PTC		AHF		PTC	
Type 0	45	22.3	11	18.3	45	37.5	11	35.4
Type 1	33	16.3	5	8.3	23	19.2	3	9.7
Type 2	124	61.4	44	73.4	52	43.3	17	54.9
	202	100%	60	100%	120	100%	31	100%

TABLE 4. RELATIONSHIP BETWEEN TYPE OF KINDRED AND PLASMA DEFECT

	Plasma Level (%)	Type of Kindred		
		0	1	2
AHF—202 patients	0 — 0.3	31	29	51
	0.4 — 1	11	3	20
	1.1 — 5	2	0	27
	6 — 20	1	1	26
PTC—60 patients	0 — 0.3	4	5	12
	0.4 — 1	1	0	9
	1.1 — 5	5	0	10
	6 — 20	1	0	13
AHF—136 carriers	45 or <	6	4	12
	50-60	9	2	26
	65 or >	15	11	51
PTC—35 carriers	45 or <	6	1	6
	50-60	1	1	7
	65 or >	3	1	9

TABLE 5. RELATIONSHIP BETWEEN AHF OR PTC LEVEL IN A CARRIER AND HER SON

	Plasma Level (%)	0 - 0.3	Sons' AHF or PTC Levels %		
			0.4 - 1.0	1.1 - 5.0	6.0 - 20
AHF—95 carriers	45 or <	10	0	3	2
	50-60	13	3	6	6
	65 or >	24	4	11	3
PTC—29 carriers	45 or <	7	1	2	2
	50-60	3	2	4	0
	65 or >	2	2	3	1

58.3 per cent of the corresponding PTC mothers were abnormal in contrast to 16.1 and 37.1 per cent of the totals in the two disorders (table 2).

AHF or PTC Levels of Carriers in Families in which Two or More Carriers Were Studied: Fig. 1 depicts all AHF kindreds in which more than one carrier was studied. When known, the AHF level is indicated. The pedigrees are abbreviated and show only the carriers and related hemophiliacs. Fig. 2 illustrates similar PTC kindreds. Low plasma AHF or PTC levels appear in

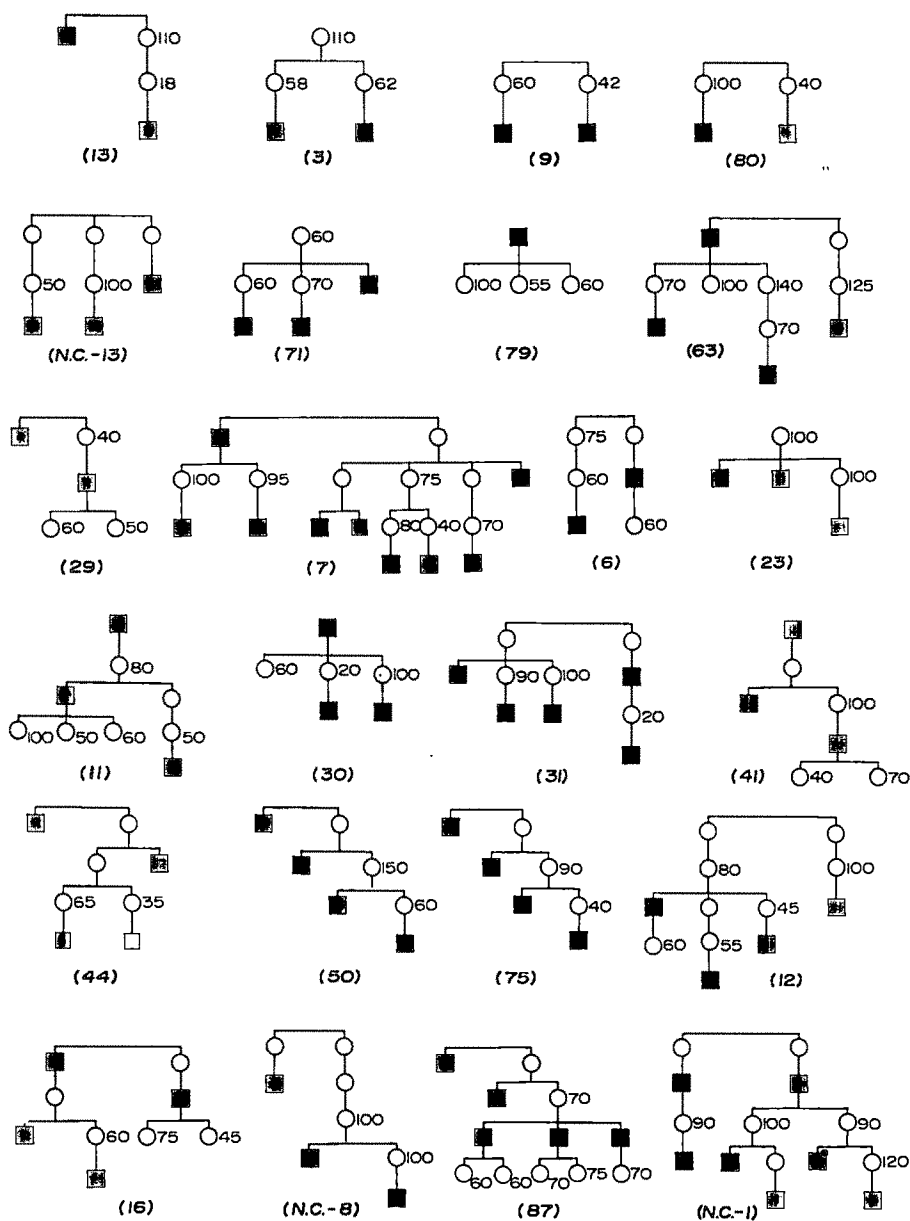


FIG. 1. AHF deficient kindreds.

random fashion and are not consistent throughout a family. Pedigree number 13 may be cited as a striking example in which one carrier had a plasma level of over 100 per cent (two tests) and her daughter had a level of 18 per cent (five tests over a five-year period with assay levels of 15 to 20 per cent).

Sex Ratio and Hemophilic Segregation: Sufficient data were available to calculate the occurrence of hemophilia and the sex distribution among the individuals of certain sibships. In an attempt to obtain unprejudiced data the sibships containing children of sisters of hemophiliacs (theoretically 50 per cent carriers) and of daughters of hemophilicas are tabulated in table 6.

DISCUSSION

Laboratory and genetic studies in two different sex-linked hemorrhagic disorders have been presented. Both hemophilia and PTC deficiency vary greatly from individual to individual in the severity of the associated bleeding symptoms and in the plasma levels of the deficient factors. As a rule the severities of the symptoms and of the plasma deficiency correlate well. There were more

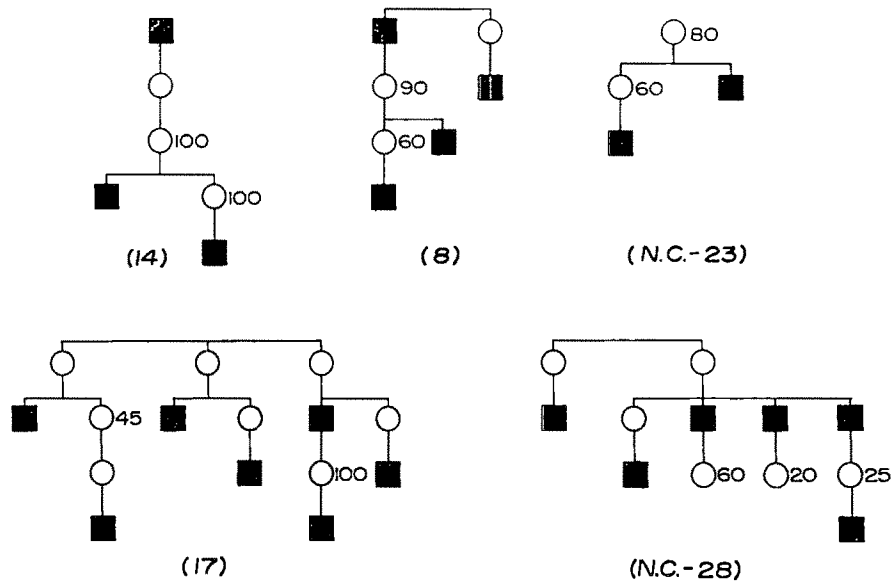


FIG. 2. PTC deficient kindreds.

TABLE 6. SEX RATIO AND INCIDENCE OF HEMOPHILIA

	No. of Sibships	Total Children	Sex		Normal	Male Bleeder	Ratio Bleeder Male
			Female	Male			
AHF sisters	205	710	342	368	246	122	(33.2%)
PTC sisters	59	160	81	79	52	27	(34.2%)
Expected ratio	—	—	1	: 1	3	: 1	(25%)
AHF daughters	61	185	91	94	45	49	(52.1%)
PTC daughters	19	103	38	65	37	28	(43.1%)
Expected ratio	—	—	1	: 1	1	: 1	(50%)

severe deficiencies of AHF than of PTC but examples ranging from mild to very severe were found in both diseases. Levels of AHF or PTC were constant on repeated testing and were usually the same in all affected members of the same family. Approximately 20 per cent (table 3) of these bleeders gave no family history of a similar disorder.

Female carriers of AHF or PTC deficiency may have normal or abnormally low plasma levels of the corresponding factor. Individual levels appeared constant on repeated testing but different carriers from the same family varied markedly as to their AHF or PTC levels. The occurrence of abnormal levels (less than 45 per cent) in carriers suggests that the abnormal genes for both AHF and PTC need not be completely recessive. Mild hemorrhagic symptoms sometimes occurred in carriers with low AHF or PTC.

Kindred were grouped in three classes: those containing only one bleeder, those containing two or more in the same sibship and those containing bleeders in multiple sibships. A greater frequency of severe deficiencies was found in patients from families of the first two types. This observation could be interpreted to mean that bleeders with milder disease survived longer and produced more offspring or that the disease itself became milder as it passed through the generations. Carriers with abnormally low levels of AHF or PTC were found in all types of kindred.

In studying the segregation for hemophilia, we could have used the sibships containing at least one hemophilic patient with statistical correction for the bias incurred by the *propositi*. There are, however, several uncertainties in doing so. The probability of encountering a hemophilic in North Carolina or Western Pennsylvania is unknown and is difficult to estimate. Whatever statistical procedure is adopted for correcting the bias, the standard error of the estimated segregation proportion is usually large so that small deviations from some expected value cannot be detected. Hence, we have decided to take advantage of the many large pedigrees in our collection and to determine the segregation ratio directly from the hemophilics' sisters' children and the hemophilics' daughters' children. No conceivable bias is involved and no correction is needed for this type of study.

The data on segregation for hemophilia as presented in table 6 have the same general features as those previously reported by two of us (Lewis and Li, 1958) except that the present calculations are based on a much larger number of observations.

In discussing the data we must distinguish very clearly between two types of segregation—one for sex and one for hemophilia (AHF or PTC, as the case may be). Sex ratio is determined by the segregation of the X and Y chromosomes of the father, while the segregation for hemophilia among male children is determined by that of the X chromosomes of the heterozygous mother. Table 6 shows that the segregation for sex is very nearly 50:50 except for PTC patients' daughters' children, among whom there are 38 females and 65 males (36.8 per cent and 63.3 per cent respectively). The chi-square value^o is 7.08, which is significant at the 0.01 level. We have no explanation for the deviation from the normal ratio.

As to the segregation for hemophilia, the most puzzling feature is that among

AHF hemophilics' sisters' male children there is a significant excess of hemophilics. One quarter of the 368 male children (92) is expected to be hemophilic while the observed number is 122. The chi-square in this case is

$$\chi^2 = \frac{(246 - 3 \times 122)^2}{3 \times 368} = 13.04$$

The same feature is also true with PTC patients' sisters' male children but the excess of bleeders is less significant ($\chi^2 = 3.55$) on account of the small number (total 79 males).

There is no previous report of or plausible explanation for the excess of hemophilics among the sons of patients' sisters. However, several possibilities may be discussed. If half of hemophilics' sisters are normal (*HH*) and half heterozygous (*Hh*), an excess of hemophilics among their children would obtain if the heterozygous sisters had larger families than the homozygous sisters, without assuming any abnormal segregation. The determination of the size of these two kinds of sibships is unfortunately not as straightforward as one might first think, because many of the heterozygous sisters do not have hemophilic sons and cannot be distinguished from homozygous sisters. Now, our data show that the segregation for sex is normal. Then, in order to bring the expected proportion of $\frac{1}{4}$ hemophilics among the sons up to the observed $\frac{1}{3}$, the size of the heterozygous sisters family must be twice as large as that of homozygous sisters, as shown in the following:

HH sisters $\rightarrow D, D, H, H.$

Hh sisters $\rightarrow D, D, D, D, H, h, H, h.$

where *D* = daughter, *H* = normal son, and *h* = hemophilic son. The known heterozygous mothers produced an average of 4.25 children, while sibships without bleeders averaged 3.0 children. Thus, an explanation on the basis of the size of families alone can hardly account for the observed excess of hemophilics.

Next let us consider the possibility that there may be more than 50 per cent carriers among hemophilics' sisters. This implies that, father being normal, their heterozygous mother produces more *h*- than *H*-gametes. By the same token, the heterozygous sisters would produce more hemophilic than normal sons. If so, the observed excess of hemophilics among hemophilics' sisters' male children would be the cumulated effect of two generations of unequal segregation favoring *h*. In order to account for the observed proportion of approximately $\frac{1}{3}$, we have to assume that the proportion of *h*-gametes produced by the heterozygous mother is of the order of $\sqrt{\frac{1}{3}} = 0.577$ instead of the usual 0.50. The standard error of the observed number (122) among a total of 368 males is 9. Therefore, an abnormal segregation ratio of 0.53 is still within the two-standard-error range. A small deviation from 0.50 like this would require a sample of thousands of children to substantiate.

An independent check on the hypothesis of unequal segregation should be provided by the data from hemophilics' daughters' male children. Table 6 shows that there are 45 normal and 49 hemophilic sons (47.9 per cent and 52.1 per cent), well within the range of normal segregation notwithstanding the slight

excess of hemophilics. Hence there is no firm support for the hypothesis of unequal segregation. We may, however, note that the number of sisters' male children (368) is almost four times as large as daughters' (94). A combination of differential family size and unequal segregation is another possibility and would reduce the requirement for deviations still further.

Finally, there remains the possibility that there is some inherent bias in our collection of pedigrees of which we are not aware. Sisters and daughters of hemophilics have been traced the same way without regard to their children's condition.

CONCLUSION

Two hundred and two patients suffering from and 136 carriers of hemophilia and 60 patients and 35 carriers of PTC deficiency were studied. Both of these hemorrhagic disorders occur predominantly in males but in each some (16 per cent in AHF and 37 per cent in PTC) of the carriers had abnormally low plasma levels of the deficient factor. Thus, these two diseases may be considered as sex-linked partially recessive. Segregation studied among hemophiliacs' sisters' children and hemophiliacs' daughters' children showed no marked deviation from the expected as to segregation for sex except among PTC patients' daughters' children. Segregation for hemophilia showed a significant excess of bleeders among patients' sisters' male children. Various explanations are considered.

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Blood Group Studies on the Family of an XX/XY Hermaphrodite with Generalized Tissue Mosaicism

ELOISE R. GIBLETT, S. M. GARTLER AND S. H. WAXMAN

*King County Central Blood Bank and the
Departments of Medicine, Genetics, and Pediatrics,
University of Washington School of Medicine,
Seattle, Washington*

THE OCCURRENCE OF TWO genetically different cell populations in the blood of human subjects is usually a transient phenomenon following blood transfusion or transplantation of hemopoietic tissue. In rare instances, permanent blood chimerism is established in non-identical twins by the transplantation of blood cells through vascular anastomoses *in utero*. Another postzygotic mechanism involves either mitotic accident or gene mutation in the blood cell precursors, the descendants of which may be distinguished by serological, cytological or biochemical techniques. Much more rarely, a prezygotic mishap is responsible for partial or complete body tissue mosaicism in which the two cell lines derive their genetic complement from different gametes. The latter process requires the incorporation of two egg nuclei into a single embryo. Dispermy is probably an additional requirement, since it is doubtful that completely haploid cell lines can be maintained in human non-germinal tissue.

In previous papers (Waxman, Gartler and Kelley, 1962; Gartler, Waxman and Giblett, 1962), we have described the clinical and cytological details of the first known human case of generalized XX/XY mosaicism due to double fertilization. This report briefly summarizes those data and presents the blood and serum groups of the patient and her family, including an anomaly in the MNSs system.

CASE REPORT

A two-year old girl of Scandinavian ancestry, admitted to the surgical service for repair of a clitoris enlarged since birth, was noted to have a small branchial cleft cyst and heterochromia simplex, the right eye being brown and the left hazel. In a culture of peripheral blood, 33 of 34 leukocytes studied had a normal chromosome count of 46. However, on karyotyping 13 cells, seven had an XX chromosome pattern and six were XY. Laparotomy revealed an apparently normal ovary on the left and an ovotestis on the right. The latter was removed

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and shown to contain ovarian follicles and seminiferous tubules. Cultures of tissue obtained from the two gonads and skin from both sides of the abdomen revealed a predominance of XX cells on the left and XY cells on the right, the ovarian and testicular portions of the ovotestis being mainly XX and XY, respectively. No XY cells were found in the tissue taken from the ovary.

These remarkable findings strongly suggested the possibility of generalized tissue mosaicism, and the subsequent demonstration of two genetically different red cell populations provided confirmatory evidence that this patient represented the product of fertilization of two egg nuclei by two spermatazoa.

METHODS AND RESULTS

Demonstration of Two Erythrocyte Populations

To provide information about the blood group phenotypes possible in their offspring, the red cells of both parents were tested in parallel with those of the patient. The results, shown in table 1, indicated that a mosaic child of this mating could potentially have red cell populations differing in five blood group systems: ABO, MNSs, P, Rh, and Kidd. Since the parents are presumably homozygous for the genes *Fy^a* and *Fy^b*, respectively, all of their offspring would be Fy (a+b+). In the Lewis system, the genes primarily affect the secretion of Lewis substance so that heterogeneity would not be expected to occur as the result of mosaicism.

The patient's red cells were agglutinated by all antisera reacting with the cells of both parents. More significantly, in those tubes containing the child's cells incubated with anti-N, anti-S, anti-s, and anti-E antibodies, there were large numbers of unagglutinated cells, a characteristic not seen in the tubes containing the parents' cells. Furthermore, the cells left unagglutinated by anti-E could be agglutinated by anti-M, anti-N and anti-s, but not by anti-S. Conversely, the cells left unagglutinated by anti-s reacted strongly with anti-S, anti-E and anti-M, but not with anti-N.

Separation of the two erythrocyte populations was accomplished by the method described by Booth *et al.* (1957), harvesting in separate tubes the cells not agglutinated by potent anti-E and anti-s agglutinins after repeated incubation, centrifugation, mixing and settling. When two essentially "pure" populations were obtained, the cells were then tested with a number of antisera. The pertinent results, shown in table 2, confirmed the suspected differences in two blood group systems, Rh and MNSs, one population reacting with anti-M, S, C, D, E, c and c, while the other reacted with anti-M, N, s, C, D, c and c antibodies.

Determination of the relative numbers of cells in each population was performed by Mrs. Marie Crookston, who tested the cells by the method of differential agglutination described by Mollison (1961), using anti-E and anti-S to distinguish one of the cell classes, and anti-s for the other. After counting over 6,000 unagglutinated cells, she concluded that the two populations were present in approximately equal numbers.

Blood and Serum Groups of the Family Members

Blood and saliva specimens were obtained from all available family members,

TABLE 1. REACTIONS OF THE PATIENT'S RED CELLS AND THOSE OF HER PARENTS WITH BLOOD GROUP ANTIBODIES

Red Cells	Antisera													
	A	A ₁	B	M	N	S	s	P	C	D	E	e	Lu ^a	Lu ^b
Father	—	—	—	+	+	+	+	+	—	+	+	+	—	+
Mother	+	+	—	+	+	—	+	+	+	+	+	+	—	—
Patient	+	+	+	+	+	+	+	+	+	+	+	+	—	—

*"Mixed fields" due to partial agglutination.

TABLE 2. REACTIONS OF THE PATIENT'S SEPARATED RED CELL POPULATIONS WITH BLOOD GROUP ANTIBODIES

Red Cells*	Antisera													
	A	A ₁	M	N	S	s	P	C	D	E	e	Le ^a	Le ^b	Jk ^a Jk ^b
Population I	+	+	+	+	+	—	+	+	+	+	+	+	—	+
Population II	+	+	+	+	+	—	+	+	+	+	+	+	—	+

*Population I prepared by selecting cells not agglutinated by anti-s
Population II prepared by selecting cells not agglutinated by anti-E.

including the patient's maternal grandparents, two male siblings and a half-brother. Through the courtesy of Dr. A. G. Steinberg, the Gm groups of the *proposita* and her parents were ascertained. The haptoglobin and transferrin types were determined by starch gel electrophoresis, as described by Smithies (1959). Tests for secretor status were performed by the method of Race and Sanger (1958). Most of the blood groups were kindly confirmed by Drs. R. R. Race and Ruth Sanger, who also tested the cells for Xg^a, the recently discovered X-linked antigen (Mann *et al.*, 1962). Unfortunately, all family members were Xg^a positive.

The findings are summarized in Fig. 1, in which the presumed genotypes are given whenever these could be ascertained, either directly or by inference. Not shown in the figure are the results obtained with antibodies against the rare antigens Mi^a, Vw, Di^a, Js^a, V, Wr^a, Be^a and Kp^a (all negative) or antibodies which identify the very common antigens Kp^b, U, Ge^a, Vc^a and I (all positive).

No further examples of mosaicism were found in this family. However, an entirely unexpected phenomenon was encountered in the MNSs system, of which the phenotypes are given in Fig. 1. The patient's mother and her parents, all being S negative, would ordinarily be homozygous for its common allele, *s*. However, the S positive erythrocytes in one of the *proposita*'s two populations, as well as those of her sibling, III-3, were not agglutinated by anti-*s*. Further studies were undertaken to elucidate this phenomenon.

Study of the Anomaly in the MNSs System

Using a saline agglutinating anti-S serum which distinguishes the cells of homozygotes and heterozygotes, titration scores of the two S-positive family members (II-3 and III-3) were obtained in parallel with control cells of known type. Both of the tested cells gave reactions typical of heterozygotes, indicating that III-3, whose cells are not agglutinated by anti-*s*, has a single dose of S. Titration scores of these cells with anti-M showed the expected single dose in II-3 and double dose in III-3, while the cells of III-2 scored as a homozygote with anti-N.

Only one agglutinating anti-*s* serum suitable for titration was available, and this antiserum was used to test the cells of the entire family on three separate occasions. Unexpected and unexplained variations in cell reactivity were unfortunately encountered, although on each occasion, the results were fairly reproducible. For example, the titration score of III-1, the patient's half-sibling, was always much lower than that of his mother and maternal grandparents, all of whom have the same phenotype, MNs(S-). Similarly, I-2 always had a lower score than her husband, I-1, but their daughter, the presumed carrier of the unusual gene, repeatedly scored higher than either parent. Although this example of anti-*s* appears to be free of other antibodies, and is usually capable of differentiating homozygotes and heterozygotes, its reactions in this study were inconclusive.

The S-negative cells of the patient's mother, incubated with anti-S antiserum, did not reduce its antibody titer. Similarly, the *s*-negative cells of sibling III-3 did not reduce the titer of anti-*s* after incubation. The blood of all S-negative family members was tested with three other examples of anti-S both in saline

and by the antiglobulin technique; no positive reactions were observed.

The results of these studies are not conclusive. They are consistent with the presence of a suppressor, a deletion or a "silent" allele such as S^u (Sanger *et al.*, 1955) inherited by the patient's mother from one of her parents and transmitted to her son, III-3 and both of the cell lines of the proposita. Its accompanying gene, M , is fully expressed.

DISCUSSION

The studies reported in this paper indicate that the phenomenon of blood group mosaicism, heretofore detected by the observation of mixed cells of groups O and A (Dunsford *et al.*, 1953; Woodruff, 1953; Nicholas, Jenkins and Marsh, 1957; Booth *et al.*, 1957) can also be disclosed by agglutinins specific for antigens in the other blood group systems. If the blood in the

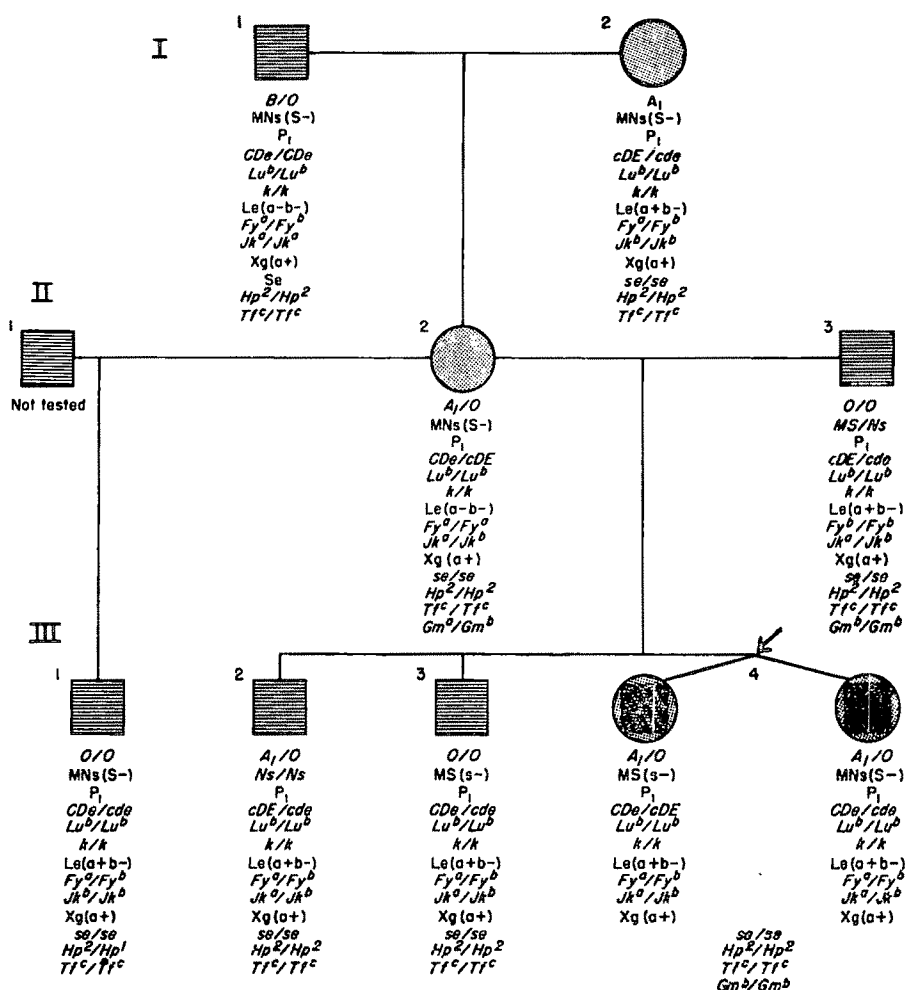


FIG. 1. Red cell and serum groups of the proposita and her family. Since it was not possible to determine which of the two red cell populations originated from precursors carrying the XX and XY chromosomes respectively, the proposita's two symbols are both given as male and female.

present case had been tested with those antibodies routinely employed for blood banking purposes (anti-A, anti-B and anti-D), the mosaicism would not have been revealed, since all of the patient's cells were A, Rh positive. Thus, the use of potent antisera of varied specificity in suspected cases may bring to light more examples of this phenomenon.

When blood mosaicism involves chromosomal abnormalities or a mixture of cells containing normal XX and XY chromosomes, karyotyping the leukocytes provides valuable information concerning the origin of a particular syndrome or the presence of blood group chimerism (Woodruff, Buckton, Fox and Jacobs, 1962). However, even in generalized mosaicism, karyotyping will be of no value if the two cell lines are normal and of the same sex. In such instances other phenomena, such as heterochromia, may provide a clue leading to an investigation of the red cell blood types. In the present case, hermaphroditism was only suggested by the enlarged clitoris, but the heterochromia simplex and demonstration of two white cell populations (XX and XY) provided sufficient evidence of generalized mosaicism to promote a thorough study of the erythrocyte and serum groups of the *proposita* and her family, and to justify abdominal exploration.

The patient's father was shown to be heterozygous at three blood group loci. The *proposita* clearly inherited both of his genes (or gene combinations), *MS* and *Ns*, in the MNSs system, as well as *cDE* and *cde* in the Rh system. Furthermore, she is known to have received both his X and Y chromosomes, and probably two paternal eye color alleles, since the father's eyes are brown and the mother's are hazel. Only dispermy can account for these findings in a euploid mosaic.

Heterozygosity in the mother was demonstrated at five loci: ABO, MNSs, Rh, Kidd and Gm. Since both parents were heterozygous at the Kidd (*Jk*) locus, the inheritance of specific *Jk* alleles could not be determined in their heterozygous children. However, in the other four instances, the *proposita* inherited only one of the two possible genes, so that there is no evidence of a double maternal contribution. As pointed out in a previous communication (Gartler *et al.*, 1962), this provides fairly good, but not conclusive, evidence that the two fertilized egg nuclei were identical, probably representing mitotic products of a post-meiotic nucleus.

The anomaly encountered in the MNSs system, although not entirely resolved, indicates that some members of this family have an unusual genetic complement which is associated with failure to express either of the alternative antigens *S* or *s*. This may represent deletion, suppression, or the presence of a third allele, *S^u*, which is postulated to be the cause of a similar phenomenon in a small percentage of Negroes (Sanger *et al.*, 1955). No examples of the critical antibody, anti-*S^u*, have yet been discovered. However, until further information is available on the complicated genetics of this blood group system, the designation *S^u* is appropriate in all cases having the serological characteristics described in this family as well as in one other Caucasian family found by Cleghorn (1962).

Armstrong, Gray, Race and Thompson (1957) described a case of true hermaphroditism in which there was no evidence of blood group mosaicism, but the patient's red cells were completely devoid of B antigen, in spite of the

presence of B substance in the saliva. The authors suggested at that time that the blood group abnormality might be genetically related to the hermaphroditism. One is similarly tempted to look for a mechanism which might correlate the anomalous MNSs blood group findings with the generalized mosaicism (and hermaphroditism) in the present case. However, the family data provide no positive evidence to support this possibility, and at least one of the patient's normal siblings shares her blood group anomaly but is not himself a mosaic.

SUMMARY

The clinical and cytological findings in a previously reported case of generalized XX/XY mosaicism in a two year old child are briefly reviewed, and the blood and serum groups of the proposita and her family are reported in detail. The presence of an anomaly in the MNSs system, known to be very rare in Caucasian subjects, is discussed. On the basis of the available data, the two phenomena encountered in this family are not genetically related in any obvious manner.

ACKNOWLEDGMENTS

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Study of a Family Possessing Hemoglobin C. Classical Thalassemia and the Abnormal Minor Hemoglobin Component A₂'

RALPH C. LEE¹ AND TITUS H. J. HUISMAN

Departments of Biochemistry and Pathology, Medical College of Georgia, Augusta, Georgia

THE MINOR HEMOGLOBIN component Hb-A₂' (or Hb-B₂), which was discovered by Ceppellini, Kunkel and Dunn (1957), is an abnormal variant of Hb-A₂. Two individuals homozygous for this hemoglobin abnormality have been discovered, one by Horton, Payne, Bridges and Huisman (1961) and another more recently, both showing a complete absence of Hb-A₂. It also has been found that Hb-A₂' differs from Hb-A₂ by the replacement of one, unknown, amino acid for arginine (Horton *et al.*, 1961). Hb-A₂' has been found in the Gullah Negro community of James Island (Ceppellini *et al.*, 1957) and in the Negro population of the U. S. A.; its incidence in the State of Georgia is estimated at about 1 per cent. Recently a family was observed (Huisman, Punt and Schaad, 1961) in which both classical thalassemia heterozygosity (to be defined as a microcytic anemia with an increased percentage of Hb-A₂) and Hb-A₂' heterozygosity were present in many members. Since the two abnormalities did not segregate, it was concluded that the genes for both abnormalities occurred on the same chromosome.

In this communication we report the results of studies of a Negro family in whose members Hb-C, thalassemia trait, and the minor Hb-A₂' occurred alone and in different combinations. The data to be presented are in accordance with the currently accepted hypothesis of allelism or close linkage of Hb-C and classical thalassemia as well as the possible allelism of Hb-A₂ and Hb-A₂'. It will be demonstrated that the biochemical and hematological abnormalities observed in one family member with both the thalassemia and Hb-A₂' heterozygosities are comparable with those described in the members of the family mentioned (Huisman *et. al.*, 1961), although the observed patterns of inheritance are different.

MATERIALS AND METHODS

Patient E. R. (I-1 Fig. 1) was admitted to the Department of Obstetrics and Gynecology of the Medical College of Georgia because of complications during pregnancy. During her stay in the hospital her hemoglobin pattern was studied by starch gel electrophoresis and was found to consist of about 75 per cent Hb-C and 25 per cent of Hb-A-like component. Some months after delivery she and her immediate family were studied. The

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family consisted of the two parents, three sons and four daughters, all except one being available for study.

Hemoglobin studies were performed by starch gel electrophoresis (Huisman, 1960). Qualitative determinations of the different Hb fractions were carried out by DEAE-cellulose chromatography (Huisman and Dozy, 1962). The alkali-resistant hemoglobin was determined using an alkali denaturation procedure (Jonxis and Visser, 1956). Hematological data were obtained using the conventional techniques.

RESULTS

Examples of results obtained by starch gel electrophoresis are illustrated in Fig. 2. In the hemolysate of the erythrocytes of the mother (I-1) two main fractions are detectable, one Hb-C and the other Hb-A, while the presence of a small amount of Hb-F is also indicated. The father (I-2) was found to be a heterozygous Hb-A₂' carrier. Three of the children (II-3; II-5; and II-7 who is not included in Fig. 2) are heterozygous Hb-C carriers. Child II-1 demonstrates three Hb components namely, Hb-A, Hb-C and Hb-A₂'. The Hb pattern of child II-2 resembles that of his father (I-2), although the amounts of Hb-A₂ and Hb-A₂' seem to be somewhat higher. The child II-4, finally, demonstrates an increased amount of Hb-A₂ while a reasonable quantity of Hb-F is also demonstrable.

Data pertaining to the hematological findings and the quantitative results of Hb analyses as carried out by chromatography and by alkali denaturation are presented in table 1. Numerous target cells were present in the smears of the Hb-C carriers and occasionally also in the blood smears of II-2 and II-4. The erythrocytes of the latter cases were also characterized by anisocytosis and poikilocytosis, while the decreased MCV values suggested a slight microcytosis. II-3 showed a mean cell volume of 67, which is unusually low for a Hb-C carrier. Remarkably enough, the data pertaining to the hematological findings in the mother (I-1) were found to be in the normal range with the exception of the presence of target cells and a decrease in the osmotic fragility of her erythrocytes.

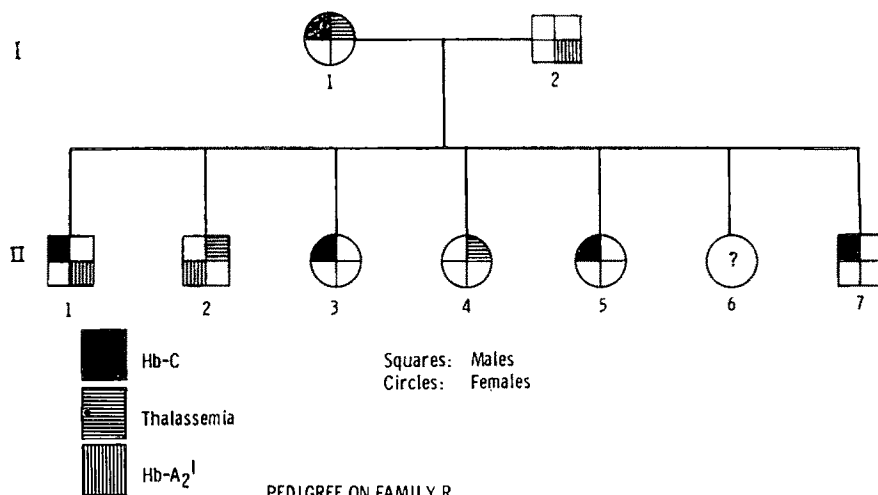


FIG. 1. Pedigree of family "R."

Quantitative studies as carried out by chromatography on DEAE-cellulose and by alkali denaturation revealed the following data: (a) About three-fourths of the Hb of case I-1 is Hb-C; a slight, but definite increase in the per cent of Hb-F was found to be present. Separation of Hb-A₂ and Hb-C was impossible. (b) The percentages of Hb-A₂ (1.25 per cent) and of Hb-A₂' (1.15 per cent) in the blood of I-2 correspond with the values obtained for heterozygous Hb-A₂' carriers (Horton *et al.*, 1961) (c) The chromatographic technique allowed the quantitative determinations of both major Hb-components Hb-C and Hb-A, and of the minor Hb components Hb-A₂ and Hb-A₂' in the blood sample of

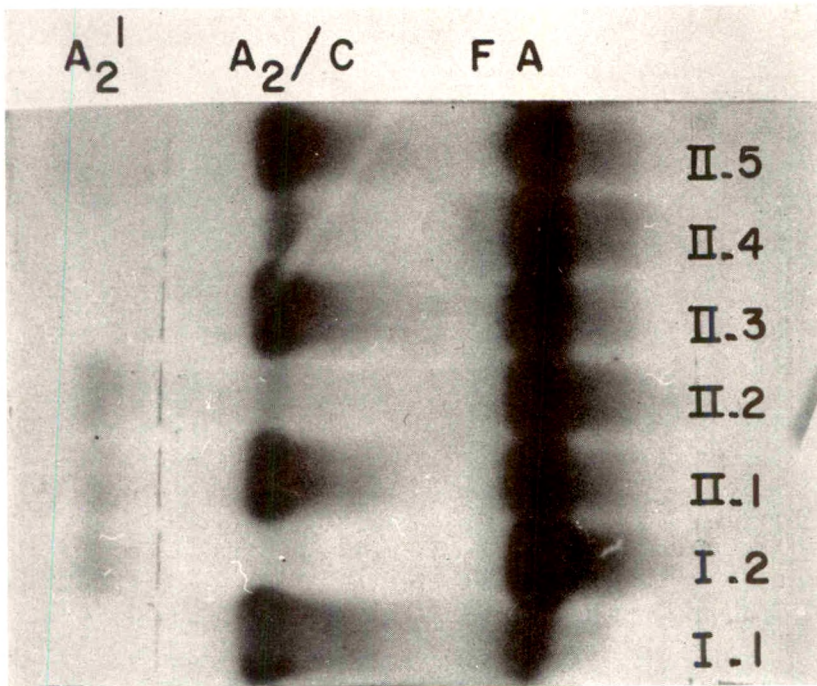


FIG. 2. Starch-gel electrophoretic patterns of the hemoglobins of several members of the family "R." The numbers indicate individuals in the pedigree (Fig. 1).

Table I : Laboratory Data

Generation No.	Age-Sex	Hb gm %	PCV %	RBC 10 ⁶ /mm ³	MCV c. μ	MCH μg	MCHC %	Hb-Pheno-type*	Hb-A ₂ † %	Hb-A ₂ '† %	Hb-F+ %	Hb-C‡ %
I-1	33-F	12.9	38	4.4	86.4	29.3	34.0	C-Thal.	---	0	5.1	75#
I-2	36-M	16.0	47	4.8	97.9	33.3	34.0	A-A; A ₂ -A ₂ ^I	1.25	1.15	< 2	0
II-1	15-M	13.6	37	4.5	82.2	30.2	36.8	A-C; A ₂ -A ₂ ^I	1.3	1.25	< 2	32.7
II-2	11-M	11.8	35	5.1	68.6	23.1	33.8	A-Thal; A ₂ -A ₂ ^I	2.7	1.85	4.6	0
II-3	8-F	12.9	35	5.2	67.3	24.8	36.8	A-C.	---	0	< 2	---
II-4	6-F	13.2	38	5.5	69.1	24.0	34.7	A-Thal.	4.1	0	8.3	0
II-5	4-F	14.0	39	4.8	81.3	29.2	36.0	A-C.	---	0	4.2	---

* Starch gel electrophoresis.

† DEAE-Cellulose Chromatography.

+ Alkali denaturation technique.

Hb-A₂ is included.

II-1. The last two minor fractions, which were eluted in front of Hb-C component, were present in amounts similar to those found for the hemolysate of erythrocytes of the father (I-2). These results suggest the presence of double heterozygosity in II-1, namely for Hb-C and for Hb-A₂'. (d) A definite increase in the percentage of Hb-F was demonstrable in the hemolysates of red blood cells of II-2 and II-4. II-4 also demonstrated an increase in the Hb-A₂ level. These data, therefore, suggest a heterozygosity for classical thalassemia in II-4. II-2 differs from II-4 only in that Hb₂' is present. The percentage of Hb-A₂', however, is definitely higher than that found for I-2 and II-1, while the amount of Hb-A₂ is also about twice the value found in heterozygous Hb-A₂' carriers. The sum of the two minor components (4.55 per cent) is comparable to the percentage of Hb-A₂ demonstrated in II-4. It is likely, therefore, that II-2 is heterozygous for classical thalassemia as well as the Hb-A₂' abnormality.

DISCUSSION

The presence of the thalassemia trait abnormality in two of her children and of the Hb-C trait anomaly in three of her children, while her husband showed heterozygosity for the Hb-A₂ variant, known as Hb-A₂', makes it likely that the probanda was heterozygous for both thalassemia and Hb-C. Mating of the probanda with a husband heterozygous for Hb-A₂', may be informative as it combines a parent heterozygous for the β -chain abnormality Hb-C (Hunt and Ingram, 1958) as well as the thalassemia gene, and a parent heterozygous for the δ -chain abnormality Hb-A₂' (Horton *et al.*, 1961). According to the current concepts of the genetic control of hemoglobin abnormalities, including thalassemia, four phenotypes are possible in the offspring of this mating. They are AC, thalassemia trait, thalassemia trait and Hb-A₂' heterozygosity, and AC and Hb-A₂' heterozygosity. All four phenotypes are present in the children (II-3, II-5 and II-7; II-4; II-2; II-1, respectively).

Of importance is the study of interactions of the gene governing the synthesis of Hb-A₂' with those of other abnormal Hb types and of thalassemia. It has been suggested (Ingram and Stretton, 1959) that in the classical type of thalassemia a hidden abnormality of the β -chains should be present. In addition, this type of thalassemia is characterized by an increase in the production of the δ -chain resulting in an excess of Hb-A₂. When the thalassemia gene and the gene for Hb-A₂' are present on the same member of a pair of homologous chromosomes, separation of the two abnormalities does not occur, and this was considered as suggestive for close linkage of the β -chain locus and the δ -chain locus (Huisman *et al.*, 1961). II-2 also shows heterozygosity for thalassemia as well as Hb-A₂'. He is similar to those cases described in the earlier study, since changes in the hematological features and the amounts of Hb-A₂ and of Hb-A₂' are identical to those previously studied. The difference, however, is the inheritance of the thalassemia gene from one parent and the Hb-A₂' gene from the other parent, implying that in this patient the two genes are present on different members of a pair of homologous chromosomes. Our family is, therefore, comparable to the one described by Ceppellini (1959) in which segregation of the thalassemia anomaly and the Hb-A₂' abnormality did occur.

SUMMARY

A study of hemoglobin patterns in members of a small Negro family is reported. From the rare mating of a Hb-C-thalassemia carrier and a heterozygous Hb-A₂' (an abnormal Hb-A₂) carrier, four different Hb-phenotypes were observed: Hb-C trait carrier, thalassemia trait carrier, thalassemia trait and Hb-A₂' trait carrier, and Hb-C trait and Hb-A₂' trait carrier.

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The authors are indebted to the Department of Obstetrics and Gynecology for referring the proposita for further study.

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Hairy Pinna of the Ear in Israeli Populations

HERMAN M. SLATIS AND AZAY APELBAUM

*Division of Biological and Medical Research, Argonne National Laboratory,
Argonne, Illinois, and Department of Zoology,
Hebrew University, Jerusalem, Israel*

AN ANALYSIS OF SUPPOSEDLY Y-linked traits by Stern (1957) and by Penrose and Stern (1958) demolished the assumption that several human characteristics are inherited in this manner. However, Dronamraju (1960) made a study in India of coarse hairs on the pinna of the ear and the new data strongly supported the earlier evidence that this trait is Y-linked (in addition to many fine hairs on the pinna, some Caucasian men have coarse hairs in this region). Although Gates has published papers confirming the Y-linkage of hairy pinna (1957, 1960; Gates and Bhaduri, 1961), these papers generally give completely Y-linked pedigrees when based on hearsay and pedigrees that contain exceptions to Y-linkage when based on observation. Sarkar *et al.* (1961) presented a number of pedigrees based largely on hearsay. Their *observed* cases include a high proportion of men who are discordant for hairy pinna although by descent they carry the same Y chromosome. Dronamraju and Haldane (1962) suggested that Sarkar *et al.* erred in stating that illegitimacy is rare in the population they studied. Lack of penetrance was also suggested to account for the observed discordances although Dronamraju (1960) had not found lack of penetrance in his series of observations.

This report is of observations of hairy pinna that were undertaken to collect data on (1) its frequency among several communities of Jews in Israel, (2) its age of onset, and (3) its mode of inheritance. For all aspects of this study one basic rule has been maintained: all observations have been made on the living subject by the senior author; no hearsay or photographic evidence was accepted. In most instances the subject's attention was diverted by a test of color vision (Ishihara charts) while a leisurely examination was made of his ears. If coarse hairs were present, their position and number were immediately recorded by sketching an ear.

Almost all grades of affected subjects were observed, from men with a single coarse hair on one ear and none on the other to men with dense growths of hair up to 2 inches in length. No one matched Tommasi's *propositus*, the first case ever reported, whose photograph was reproduced by Sarkar *et al.* (1961). It was decided that a man should be classified as "affected" if he had at least several coarse hairs on the top or side of the ear. There is a good deal of bilateral symmetry in the degree to which a man is affected, even including one man with one malformed ear. The scale devised by Sarkar *et al.* for describing

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intensity of ear hairiness is not applicable in this study. In Israeli populations hair frequently occurs at the top of the ear, in addition to its occurrence in the sulcus at the side of the ear, which is the common site among Indians. At each of these two sites the hair can be classed as light or heavy in quantity. Growth of a few hairs on the ear lobe appeared to be independent of hair on the pinna and toward the end of the study it was not recorded. Dense growths of hair were not seen on the lobe except as an extension of the hair at the side of the ear. Hairs around the external auditory meatus were assumed to be an independent phenomenon, but observations were made to determine the validity of this assumption.

Following Dronamraju (1960), affected men are referred to by the symbol *He*, unaffected men by +. For a Y-linked character, considerations of dominance relationships are not applicable.

RESULTS

Population Studies of Hairy Pinna

In the Indian families observed by Dronamraju (1960), penetrance of hairy pinna probably is complete by 20 years of age. The age of onset is much later in Israeli populations. Of 373 largely unrelated men between 18 and 29 years old (most of whom were university students between 18 and 23), only four were affected. A total of 495 unrelated men at least 30 years old was observed in hospital wards and clinics. Separated by age into decades, it appears that the frequency of hair on the pinna increases throughout adult life (table 1), or perhaps to about age 60. A further separation was made into communities of origin. The assigned origin is the father's community in the few instances where this differed from that of the mother. These communities are (1) Ashkenazi, who are persons from central and eastern Europe, (2) Iraqi-Iranian, who are from Iraq, Iran, and Turkey, and in this sample are largely men from Baghdad, (3) North African, which excludes Egypt, with most of the observed men from Morocco, (4) Yemenite, (5) all other communities, plus a few men who failed to state their community, usually because their parents had been born in Palestine. This grouping is based on that employed by Kalmus *et al.* (1961). During this study 10 Arabs over 30 years old were observed and two of them were affected. They are not recorded in the table.

Small numbers and the variable age of onset of the trait would almost

TABLE 1. FREQUENCY OF HAIRY PINNA BY COMMUNITY OF ORIGIN AND AGE

Age Group	All Men				Ashkenazi		Iraqi-Iranian		North African		Yemenite		Other	
	+	He	Total	Proportion He	+	He	+	He	+	He	+	He	+	He
18-29	369	4	373	.011	226	3	47	0	27	0	7	1	62	0
30-39	81	5	86	.058	30	1	23	2	12	0	4	2	12	0
40-49	60	11	71	.155	36	4	12	2	5	1	2	0	5	4
50-59	99	14	113	.124	68	6	16	2	9	3	1	1	5	2
60-69	99	29	128	.227	57	12	23	10	7	2	7	0	5	5
70-99	72	25	97	.258	43	13	13	9	7	1	5	1	4	1

+ = normal
He = hairy pinna

preclude useful tests of significance of frequency differences among these communities. A comparison of the numbers of affected and unaffected men at least 60 years old in the Ashkenazi and Iraqi-Iranian communities just fails to show a significant difference (χ^2 of 3.62, 1 degree of freedom, P greater than .05).

In Fig. 1 the position and quantity of hair on the pinna have been recorded for each affected man observed in the population studies, except for one Ashkenazi student born in 1939 whose record has been lost. All observations were made within a two-month period at the end of 1961 and beginning of 1962, so that each man's age is that attained in 1961. These data suggest that the trait is first visible either at the side or the top of the ear and it progresses variably with age. In this figure the two common population groups have been distinguished. Among affected Ashkenazis, eighteen have hair only at the top of the ear while seven have hair only at the side, in contrast with affected Iraqi-Iranians, of whom two have hair only at the top while eleven have it only at the side. This difference is highly significant (χ^2 is 8.84, 1 degree of freedom, P less than .01). During the examinations it appeared that the practice of cutting this ear hair is most common in the Ashkenazi community. This practice can more easily mask the presence of hair at the top of the ear than hair at the side. Therefore, the true difference in site of hair on the pinna may be a bit greater than that reported here due to a few tonsorial phenocopies of normal among the Ashkenazis.

Family Studies of Hairy Pinna

In addition to the propoiti observed in the population study, a number of friends and men known through mutual friends were found to have hairy ears.

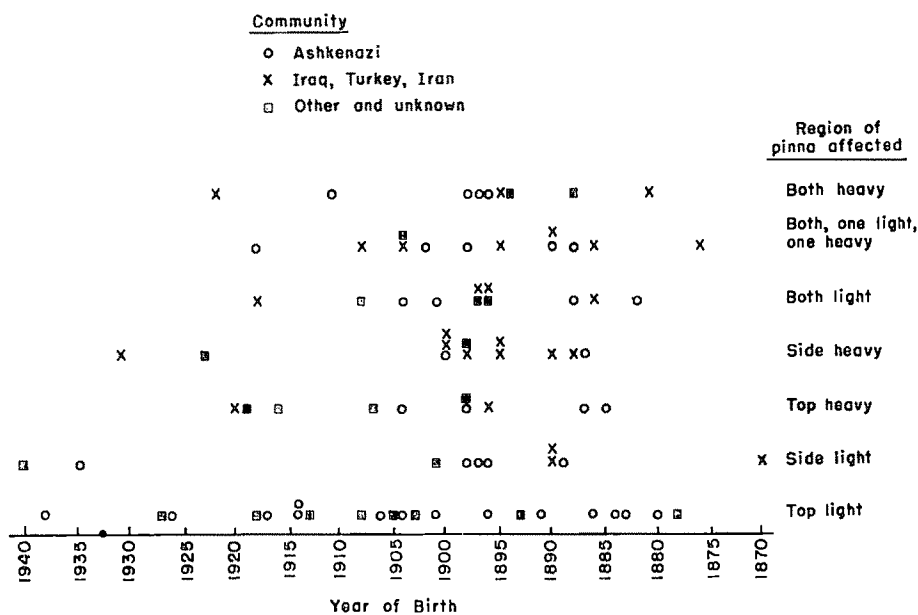


FIG. 1. The distribution of affected men in the population by condition and age.

Ten of these became probands in family studies, but since they were not observed in the course of the population study, they are not recorded in Fig. 1. In addition, it was possible to locate appropriate relatives for 16 of the 88 affected men in the population study. Because of the plan to test the theory of Y-linkage, only men who carried the same Y chromosome as a proband were examined: that is, the 67 relatives who were observed were men who were related to a proband through the male line.

Whenever possible, appropriate relatives of probands were examined. In no instance was the follow-up dependent upon remarks made concerning the presence or absence of the trait. Most subjects did not know that hairy pinna was the condition under investigation. Further follow-up was not dependent upon the outcome of the observation of closer relatives (*e.g.*, first cousins were observed whenever possible, regardless of whether or not the father and uncle were affected). Observations were made of 10 fathers, 8 uncles, 18 brothers, 8 first cousins, 15 sons, 5 nephews, and 3 grandsons of probands, all at least 23 years old. Details of these observations are given in the appendix. Each man is listed after his father (if the father was observed) and, with this rule, there are no ambiguously defined relationships in the table. The distribution of these relatives, by the degree to which they have the trait and with the two major communities distinguished as in Fig. 1, is shown in Fig. 2.

Because of the varied age of onset, one would not expect all men carrying the gene to show the hairy pinna trait. The analysis will begin with the assumption that penetrance is complete among the men in the oldest age group. "Apparent penetrance" can be defined as the ratio between the frequency of affected men in a given age group and the .258 frequency that was observed among men

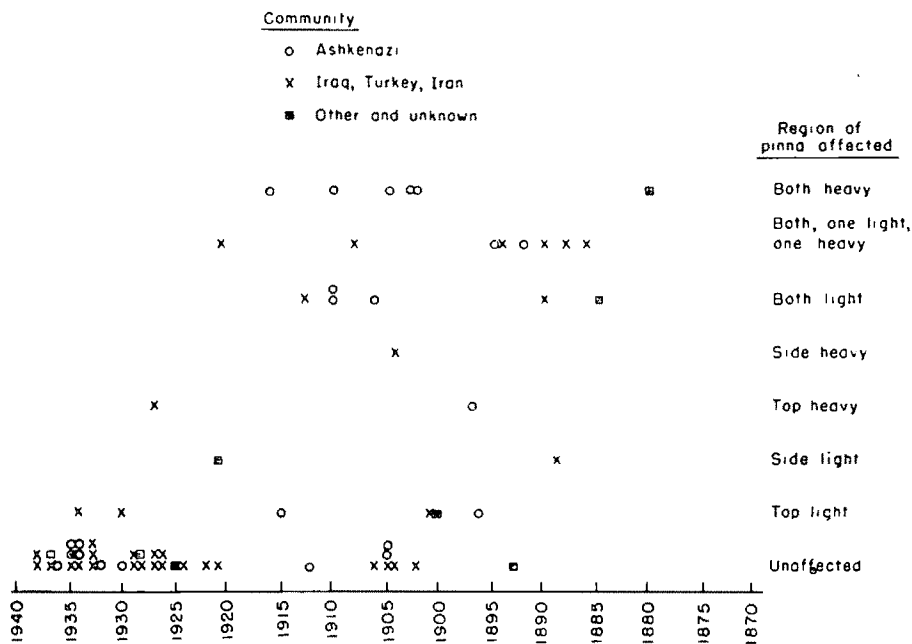


FIG. 2. The distribution of relatives of affected men by condition and age.

at least 70 years old (table 2). Accidents of sampling and biases in the sampling have clearly affected the figures for apparent penetrance. To correct this, an "adjusted penetrance" has been computed. The adjusted penetrance is roughly equivalent to a line of best fit for the apparent penetrance. Because the youngest age group was biased by a large sample of men around age 20, the apparent penetrance of .04 is undoubtedly too low. Therefore, the 18-29 age group has been assigned an adjusted penetrance of .10. The next decade has been assigned an adjusted penetrance of .20, and an additional .20 has been added to this value for each subsequent decade of life.

The right-hand portion of table 2 indicates the number of relatives observed at each age level and the number affected among them. On the theory of Y-linkage, the expected number of affected men (number of relatives times adjusted penetrance) is seen to agree closely with the number observed, and a test of the difference yields a χ^2 value of 0.11. Of course, many other estimates of adjusted penetrance are possible, but any reasonable estimate of the penetrance based on the population study gives good agreement with the number of affected relatives observed at each age level and with the total number of affected relatives.

If penetrance is not complete in the oldest age group, the estimates of the adjusted penetrance will be too high. Among these relatives, all seven of the men over 70 and seven of the eight men in their sixties showed the trait. On the theory of Y-linkage, this is in extremely good agreement with the assumption that penetrance is complete or nearly complete among older men.

Although the data agree with the theory of Y-linkage, other possible modes of inheritance must also be investigated. The analysis of the data on the theories of autosomal inheritance will begin with the assumptions that are most favorable to the acceptance of these theories. One of these assumptions is that penetrance is complete by some given age, which here is taken as age 40.

A breakdown by relationship, whether or not affected, and whether or not at least 40 years old, is given in table 3. It can be shown that if a trait is sex-limited to males and is inherited as a simple autosomal dominant condition with gene frequency p , the probability of an affected father of an affected man is $(1 + p - p^2)/(2 - p)$. This formula was derived from simple mendelian considerations, but for greater ease of derivation one can also use the ITO method as described by Li and Sacks (1954) and Li (1955, pp. 34-39).

TABLE 2. FREQUENCY OF AFFECTED RELATIVES OF PROPOSITI BY AGE

Age Group	Population Study			Family Study		
	Proportion Affected	Apparent Penetrance	Adjusted Penetrance	N	Affected	Expected if Y-linked
18 - 29	.011	.04	.10	16	1	1.6
30 - 39	.058	.22	.20	14	2	2.8
40 - 49	.155	.60	.40	7	5	2.8
50 - 59	.124	.48	.60	15	9	9.0
60 - 69	.227	.88	.80	8	7	6.4
70 - 99	.258	1.00	1.00	7	7	7.0
				67	31	29.6

These methods must first compute the frequency of fathers in each genotype for propoiti in each genotype. One then computes the frequency of affected fathers of affected men among all fathers of affected men. The same formula applies to the probability that the son of an affected man will be affected. Similarly, the formulas for affected men in other degrees of relationship to affected propoiti are:

Affected brothers: $(4 + 5p - 6p^2 + p^3)/4(2 - p)$

Affected uncles, nephews, grandfathers, grandsons:

$$(1 + 5p - 5p^2 + p^3)/2(2 - p)$$

Affected first cousins: $(1 + 13p - 13p^2 + 3p^3)/4(2 - p)$

Among the older men the prevalence of hairy pinna is about .25. At this prevalence the frequency of an autosomal dominant gene for the trait would be about .14. The expected frequencies and expected numbers of affected men at least 40 years old in each class of relationship are given in table 3. There is reasonably good agreement for all men at least 40 years old except for the uncles, too many of whom are affected. A χ^2 analysis of the total number of affected relatives shows that the difference between the 20.8 affected relatives expected and the 28 observed is significant (χ^2 of 5.69, 1 degree of freedom, P less than .05). On the hypothesis that the trait might be caused by a simple autosomal recessive gene with sex-limited expression, the gene frequency that gives a prevalence of .25 is .50. Derived in the same way, a set of very different formulas gives the expected frequencies that are listed for men at least 40 years old. The discrepancy between the observed and expected number of affected relatives is highly significant for the men over 40 years old (χ^2 of 10.8, 1 degree of freedom, P less than .01).

These analyses have shown significant excesses of affected relatives of propoiti for theories both of autosomal dominant and autosomal recessive inheritance. These significant results were obtained under the assumptions most favorable to the theories of autosomal inheritance: (1) that the gene frequency is high, (2) that the penetrance is high, and (3) that the frequency of affected men does not change after age 40.

On assumption (1), if the estimate of the gene frequency was too high (because it was based on the high frequency among men at least 70 years old rather than on all men at least 40 years old), the expected frequency of affected relatives would be lower than that computed in table 3, and the

TABLE 3. HAIRY PINNA IN RELATIVES OF PROPOSITI

Relationship	Under 40		Over 40		Dominant Inheritance		Recessive Inheritance	
	+	He	+	He	Expected Frequency	Expected Number over 40	Expected Frequency	Expected Number over 40
Fathers	0	0	1	9	.60	6.0	.50	5.0
Sons	8	0	3	4	.60	4.2	.50	3.5
Brothers	7	0	4	7	.62	6.8	.56	6.2
Nephews	5	0	0	0	.43	—	.37	—
Uncles	0	0	0	8	.43	3.5	.37	3.0
Grandsons	3	0	0	0	.43	—	.37	—
First cousins	4	3	1	0	.35	0.3	.31	0.3
	27	3	9	28		20.8		18.0

TABLE 4. INTERRELATIONS OF VARIOUS CHARACTERISTICS

Age		Hairy Tragus		Chest Hair		Head Hair		Hair on the Nose		Color Vision		
	30-59	60-99	Absent	Present	Absent	Present	Complete	Balding	Not Observed	Present	Normal	Impaired
•	240	171	270	141	52	127	163	117	398	13	348	30
+	30	54	24	60	6	28	23	29	71	13	68	7
Pinna	13.6*		38.3*		1.3		2.9		18.8*		0.0	
χ ²	30-59	193	77	41	115	143	81	263	7	230	27	
	60-99	101	124	17	40	65	206	19	186	10		
	χ ²		34.9*		0.1		16.1*		7.3*		3.6	
	Hairy tragus		Absent	94	46	139	82	285	9	251	20	
Chest hair	Present		12	61	47	64	184	17	165	17		
	χ ²		5.7†		11.8*		5.9‡		0.3			
	Absent		39	10	58	0	53	1				
	Present		77	63	143	12	129	19				
Head hair	χ ²		8.3*		3.4		4.2‡					
	Complete		177	9	169	8						
	Balding		140	6	115	19						
	χ ²		0.0		7.8*							
Hair on the nose	Not observed		394		36							
	Present		22		1							
	χ ²		0.1									

*P less than .01
 ‡P between .05 and .01

difference between expected and observed would be greater and more significant under either autosomal theory. (2) If the penetrance is less than complete among the oldest men, the estimate of the gene frequency is too low. However, the frequencies of affected relatives would be even lower than those computed in table 3 according to the simple theories of autosomal inheritance. The discrepancies between observed and expected would again be increased. (3) The expected frequencies of affected relatives given in table 3 are the genotypic frequencies uncorrected for late age of onset. This correction would greatly lower the expected frequencies of affected relatives and would enormously increase the discrepancies observed. Thus, there are serious departures from expectation on either theory of autosomal inheritance. Any complex theory of autosomal inheritance would also fail to provide a satisfactory explanation of the high frequency of affected relatives of affected men.

A simple theory of X-linkage of this trait would predict that the relatives listed in table 3 (except for brothers) would have the same frequency of the trait as that found in the general population. The high frequency of affected relatives clearly rules out this possible mode of inheritance.

The Interrelationships Between Various Characteristics of the Population Under Examination

At the same time that examinations were made of the hair on the pinna of the ear, various other traits were recorded for each subject. Analysis of these data will be limited to men at least 30 years old. The observed traits always included the quantity of hair on the tragus, which is the protuberance of the ear just in front of the external auditory meatus. A test of color vision was almost always performed. When clothing permitted (hats are commonly worn, even indoors, while open shirts often exposed much of the chest), hair growth on the head and chest were noted. The presence of coarse hairs on the outer surface of the nose was not consciously looked for in all persons, but was recorded if observed. The interrelationships among these traits are presented in table 4. For all the observed characteristics, greater information is available. Thus, age is known by year of birth (although some men could only estimate their age); both the amount of hair on the tragus and on the chest were characterized in four grades; the head hair was characterized in numerous grades; nose hair was sketched to record both position and quantity; and impaired color vision is describable in various ways. The number of categories into which these men might be distributed is further complicated by the variety of countries from which they or their ancestors had migrated. All these traits probably have frequencies that differ according to the country of origin.

The twenty-one comparisons made in table 4 include nine that are highly significant (P less than .01) and another three that are significant (P between .05 and .01). Only the nine highly significant relationships will be discussed, and these are indicated by numbers in parentheses. The relationship between hairy pinna and age (1) has already been discussed, and it is not of great interest here that among men over 30, age is correlated with the presence of hair on the tragus (2), hair on the nose (3), and the degree of baldness (4). There is a strong correlation between hairy pinna and hairy tragus (5). Part, but not

all, of this association can be ascribed to the increased frequency of each trait with increasing age. Some persons have been classed as intensely affected for one of these forms of ear hair and unaffected for the other. Within families there is no apparent association between hairy pinna and hair on the tragus. It does not appear that these two traits are part of the same phenomenon in the way that hair at the top of the pinna seems to be related to hair at the side of the pinna.

The inadequate method of observation of hair on the surface of the nose makes its association with hairy pinna (6) suggestive, but not definite. The association between baldness and impaired color vision (7) may be in an accident of sampling. No physiological basis for this relationship can be postulated. It is known that color blindness is non-random with respect to ancestry among these communities (Kalmus *et al.*, 1961), and non-randomness is also undoubtedly present with respect to baldness (*e.g.*, bald Yemenites are extremely rare). Despite this, it is not likely that the joint non-random distribution of these two traits explains their apparent association.

The association of baldness and hairy tragus (8) is strongly affected by the tendency of each of these traits toward increased frequency with increasing age. A relationship has also been found between chest hair and baldness (9). Data on this correlation have been collected more thoroughly in an American population (Slatis, Hirsch, and Finkel, in preparation).

DISCUSSION

Prior to this survey, population studies of hair on the pinna of the ear had only been carried out among the men of India and Ceylon (Dronamraju, 1961, Sarkar *et al.*, 1961) but this may be a common trait among all groups of Caucasian men. Hairy pinna is less obvious among affected Europeans than among Indians because (1) in men with brown, blond, or red hair, the hairs on the pinna may be unpigmented, (2) the site of these hairs is less often on the side of the ear, which is the most easily observed position, (3) age of onset is later, which reduces the frequency of affected men at a given gene frequency, and (4) there is probably a higher frequency of cutting of this hair. The men observed in this study were largely dark-haired, and so had pigmented hair on the ears, often when the head hair had turned completely white. There was only a small frequency of men who cut the hair for cosmetic reasons. Observations in the U. S. suggest that the frequency and site distribution of hairy pinna among men of English and Scottish ancestry may be similar to the values obtained among the Ashkenazi community. It appears that moderate frequencies of affected men occur in all European nationality groups.

The late age of onset of this trait has made it difficult to determine its mode of inheritance. This study tested the simple assumption that hairy pinna is Y-linked, and observations were limited to men carrying the same Y chromosome as an affected propositus. If one takes into account the reduced penetrance before the age of 70, there is exceptionally good agreement between the observations and the expectations on the basis of Y-linkage. An assumption of autosomal inheritance either as a dominant or a recessive sex-limited trait gives

unsatisfactory results with far too many affected relatives of affected men. Thus, it would appear that hairy pinna is Y-linked and has a high degree of penetrance at advanced ages.

Hairy pinna occurs at a high frequency among young men in India (personal observation, H. M. S.). Comparison of the results of Dronamraju (1960) with those of Sarkar *et al.* (1961) suggests that the latter studied a community that includes the late-onset genotype found in the Israeli populations, while the former studied a community with only the genotype for onset early in adult life. It is probable that the early-onset form is characteristic of most of India. On the whole, Iraqi-Iranian men tend to be affected at the side of the ear while Ashkenazi men tend to be affected at the top of the ear. The Iraqi pattern is similar to the most common pattern observed among Indians.

SUMMARY

Observations of just under 900 men have established that the potential for hair on the pinna of the ear is present in about a quarter of Israeli Jews, but that the age of onset is variable and late. The data agree with the hypothesis that this trait is determined by a gene on the Y chromosome. The earliest age of onset of the trait is about 20, but penetrance does not appear to be complete until very late in life.

Among Ashkenazi men this gene seems to occur most often as an allele that causes hair to start growing at the top of the ear. Among Iraqi-Iranian men the most common allele seems to cause hair to start growing at the side of the ear. Among Indians there may be a hairy pinna allele that causes an early onset of the trait. The frequency of the trait among all men of European ancestry is much higher than previously suspected.

Observations were also made of hair on the tragus of the ear, hair on the head, hair on the surface of the nose, hair on the chest, and color vision. Some interrelations were found between these traits, but none were striking. Part of the observed relationships were due to separate correlations with age. Correction for the age effect still leaves a correlation between hair on the pinna and hair on the tragus, but hair at these two regions on the ear appears to be due to basically independent events.

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APPENDIX

Family	Origin	Relationship to propositus	Born	Hairy pinna	
				Top	Side
1.	Iraq	Self	1898	0	Heavy
		Son	1924	0	0
2.*	Iraq	Father	1886	Heavy	Light
		Brother	1904	0	0
		Brother	1921	0	0
		Self	1926	Light	Light
		Brother	1929	0	0
		Brother	1938	0	0
		Uncle	1888	Heavy	Light
		Cousin	1926	0	0
		Cousin	1930	Light	0
		Cousin	1934	Light	0
		Cousin	1938	0	0
		Uncle	1894	Heavy	Light
		Cousin	1927	Heavy	0
		Uncle	1901	Light	0
		Cousin	1937	0	0
3.	Iraq	Self	1876	Light	Heavy
		Son	1902	0	0
		Grandson	1928	0	0
		Grandson	1933	0	0
		Grandson	1935	0	0
		Son	1904	0	Heavy
		Son	1906	0	0
		Son	1908	Light	Heavy
		Son	1913	Light	Light
		Son	1926	0	0
4.	Iraq	Father	1889	0	Light
		Self	1922	Heavy	Heavy
		Brother	1927	0	0
5.	Iraq	Self	1881	Heavy	Heavy
		Brother	1890	Light	Light
		Nephew	1922	0	0
6.	Iraq	Nephew	1933	0	0
		Self	1895	0	Heavy
		Brother	1905	0	0
7.	Iraq	Self	1895	Heavy	Light
		Son	1927	0	0
8.*	Iraq	Self	1918	Heavy	Light
		Brother	1921	Light	Heavy
		Brother	1934	0	0

9.	Iraq	Brother	1890	Light	Heavy
		Nephew	1929	0	0
		Nephew	1934	0	0
10.	Yemen	Self	1895	Heavy	Heavy
		Father	1880	Heavy	Heavy
		Self	1927	Light	0
11.	Yemen	Brother	1928	0	0
		Father	1893	0	0
		Self	1940	0	Light
		Uncle	1900	Light	0
		Cousin	1937	0	0
12.	Bukhara	Brother	1885	Light	Light
		Nephew	1925	0	0
		Self	1894	Heavy	Heavy
13.	Morocco	Son	1921	0	Light
		Self	1898	0	Heavy
		Son	1935	0	0
14.	E. Europe	Self	1884	Light	Light
		Son	1905	0	0
15.*	Poland	Father	1910	Light	Light
		Self	1933	Light	Light
16.*	Poland	Father	1905	Heavy	Heavy
		Self	1938	0	Heavy
17.*	Poland	Father	1902	Heavy	Heavy
		Self	1922	Heavy	Heavy
		Uncle	1906	Light	Light
		Uncle	1916	Heavy	Heavy
18.	Poland	Self	1900	0	Heavy
		Son	1930	0	0
		Son	1934	0	0
		Brother	1910	Heavy	Heavy
19.*	Poland	Father	1903	Heavy	Heavy
		Self	1930	Heavy	Light
		Brother	1935	0	0
20.*	Poland	Father	1895	Light	Heavy
		Self	1929	Light	Heavy
		Brother	1934	0	0
21.*	Poland	Brother	1915	Light	0
		Self	1918	Light	0
22.	Lithuania	Self	1896	0	Light
		Son	1936	0	0
23.	Romania	Self	1901	Light	0
		Son	1932	0	0
24.	Romania	Uncle	1892	Heavy	Light
		Father	1896	Light	0
		Self	1938	Light	0
25.*	Hungary	Self	1926	Heavy	0
		Uncle	1897	Heavy	0
26.*	Germany	Self	1902	0	Light
		Brother	1910	Light	Light
		Brother	1912	0	0
		Cousin	1905	0	0

*These propositi were not ascertained through the population study.

Estimation of Recessive Gene Frequencies from Data on Consanguineous Marriages

MASATOSHI NEI

Division of Genetics, National Institute of Radiological Sciences, Chiba, Japan

DAHLBERG'S (1948) formula for estimating the frequency of recessive genes in man ignores information from consanguineous marriages other than those of first cousins. This limitation was removed by Kimura (1958), who developed a maximum likelihood estimation, but his method requires a tedious iterative solution. The formulas here presented are free of these two criticisms.

LARGE POPULATIONS

In small populations certain types of consanguineous marriages are expected to occur with sufficiently high frequencies even under random mating, but these frequencies rapidly decrease as the size of population increases (*cf.* Dahlberg, 1929; Nei and Imaizumi, 1963). We thus assume that the population under investigation is so large that all types of consanguineous marriages expected under random mating are negligible and therefore the consanguineous marriages observed are all due to non-randomness of mating.

Following Kimura's (1958) terminology, let C_i be the frequency of marriages between subjects related with coefficient of parentage f_i where $f_0 = 0$ for unrelated subjects, $f_1 = 1/16$ for first cousins, $f_2 = 1/32$ for first cousins once removed or half-first cousins, $f_3 = 1/64$ for second cousins, etc. ($i = 0, 1, 2, \dots, r$), and

$$\sum_{i=0}^r C_i = 1$$

Here consanguineous marriages having $f_i > 1/16$ are neglected because of their low frequencies in human populations.

In the offspring of parents related with f_i the frequency of recessive homozygotes (P_i) for a trait controlled by a pair of genes is expected to be

$$P_i = q^2 + q(1 - q)f_i$$

where q is the frequency of the recessive gene. Thus, the total frequency of recessive homozygotes (P) in the population is

$$P = \sum_{i=0}^r C_i P_i = q^2 + q(1 - q)f$$

where f is $\sum_{i=0}^r C_i f_i$, *i.e.*, the mean inbreeding coefficient in the offspring generation. The proportion of recessive homozygotes whose parents are related with f_i is then given by

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$$k_i = \frac{C_i P_i}{P} = \frac{C_i [q + (1-q) f_i]}{q + (1-q) f}$$

Hence,

$$\begin{aligned} \sum_{i=1}^r k_i &= \frac{q \sum_{i=1}^r C_i + (1-q) \sum_{i=1}^r C_i f_i}{q + (1-q) f} \\ &= \frac{Cq + (1-q)f}{q + (1-q)f} = K \end{aligned}$$

where C is the total frequency of consanguineous marriages and K is the proportion of all recessive homozygotes whose parents are related. The gene frequency is therefore obtained directly from the above equation. That is,

$$q = \frac{f(1-K)}{f(1-K) + K - C}$$

Assuming that C and K are distributed as binomials and f is constant, the variance of q in large samples is approximately given by

$$\sigma_q^2 = \frac{(1-K)(1-C)f^2}{[f(1-K) + K - C]^4} \left[\frac{K(1-C)}{n_k} + \frac{C(1-K)}{n_c} \right]$$

where n_c and n_k are the total numbers of observations on which C and K are based respectively.

If $C_i = 0$ for $i \geq 2$, the formula for q reduces to Dahlberg's and becomes equivalent to Kimura's.

Numerical example Furusho (1957) obtained the following data for congenital deaf mutism in Japan.

Parental relation	Unrelated	First cousins	1½ cousins	Second cousins	2½ cousins
Frequency	879	484	26	68	5
k_i (%)	(60.12)	(33.11)	(1.78)	(4.65)	(0.34)
	$C = 0.0862$		$f = 0.0046$		

If we assume that congenital deafness is caused by an autosomal recessive gene (cf. Chung *et al.*, 1959 for detail), q is obtained as follows:

$$q = \frac{0.0046 \times 0.6012}{0.0046 \times 0.6012 + 0.3988 - 0.0862} = 0.00877$$

This value is smaller than that obtained by Dahlberg's formula (0.01063) and larger than Kimura's (1958) estimate (0.00761). The standard error of our estimate is calculated to be 0.000586, while in Kimura's method it is 0.00035.

COMPARATIVELY SMALL POPULATIONS

In populations which are small but not so small as to make random genetic drift important, we represent the frequencies of consanguineous marriages by C_i as before and those which are expected to occur under random mating by c'_i ($i = 0, 1, 2, \dots, r$). The excess of C_i over c'_i is denoted by c_i , so that $C_i = c'_i + c_i$. Thus,

$$\sum_{i=0}^r c'_i = 1 \qquad \sum_{i=0}^r c_i = 0$$

The frequency of recessive homozygotes in the offspring of parents related with f_i is given again by

$$P_i = q^2 + q(1-q)f_i$$

The excess of recessive homozygote frequency over q^2 due to inbreeding is

$$\Delta P = \sum_{i=0}^r c_i [q^2 + q(1-q)f_i] = q(1-q)f$$

where f is $\sum_{i=0}^r c_i f_i$. Now, the total frequency of recessive homozygotes is

$$P = q^2 + q(1-q)f$$

On the other hand, under random mating it is expected that

$$\begin{aligned} q^2 &= \sum_{i=0}^r c'_i [(q^2 - \Delta q^2) + q(1-q)f_i] \\ &= q^2 - \Delta q^2 + q(1-q)f' \end{aligned}$$

where f' is $\sum_{i=0}^r c'_i f_i$. Hence,

$$\Delta q^2 = q(1-q)f'$$

The proportion of the recessive homozygotes whose parents are related with f_i is therefore given by

$$\begin{aligned} k_i &= \frac{C_i[q^2 - q(1-q)f' + q(1-q)f_i]}{q^2 + q(1-q)f} \\ &= \frac{C_i[q + (1-q)(f_i - f')]}{q + (1-q)f} \end{aligned}$$

and

$$\sum_{i=1}^r k_i = \frac{Cq + (1-q)[f + f'(1-C)]}{q + (1-q)f} \equiv K$$

From this equation we have

$$q = \frac{f(1-K) + f'(1-C)}{f(1-K) + f'(1-C) + K - C}$$

The comparison of this formula with the previous one shows that if the consanguineous marriages expected under random mating are neglected in spite of their sufficiently large values, the gene frequency is expected to be under-estimated. The frequencies of consanguineous marriages under random mating are the function of mean and variance of family size and age differences between mates and sibs. They are obtainable under specified conditions by the formulas developed by Nei and Imaizumi (1963), and in general they are sufficiently large for a population of size less than 1,000.

Further, it may be noted that if the frequencies of consanguineous marriages are so small as is expected under random mating the formula given above reduces to

$$q = \frac{f'(1-C)}{f'(1-C) + K - C}$$

If there are no consanguineous marriages other than first cousins, this becomes

$$q = \frac{c'_1 - c'^2_1}{16k_1 - 15c'_1 - c'^2_1}$$

while Dahlberg's formula equivalent to this is

$$q = \frac{c'_1}{16k_1 - 15c'_1}$$

The difference between our formula and Dahlberg's is due to the fact that in our formula a finite correction has been made for q^2 .

SUMMARY

Dahlberg's (1948) formula for estimating the recessive gene frequency in man is extended to cases where information is available on consanguineous marriages other than those of first cousins.

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Estimation of Mutation Rate in Rare Recessive Traits

MASATOSHI NEI AND YOKO IMAIZUMI

Division of Genetics, National Institute of Radiological Sciences, Chiba, Japan

THE MUTATION RATE in rare recessive traits of man is usually estimated on the assumption that gene frequencies in the population under investigation are in equilibrium with respect to the balancing forces of mutation and selection. Geneticists such as Haldane (1949) and Neel (1952) have criticized the validity of this assumption, pointing out that there has almost certainly been a decrease in the inbreeding coefficient in the past resulting from population movement and changing social customs, which must have led to the breakdown of population equilibrium. This seems to be especially true in Europe. In Japan there are reasons to believe that the inbreeding coefficient has been fairly constant until quite recently but at present is steadily decreasing (Neel, Kodani, Brewer and Anderson, 1949; Sugiyama and Schull, 1960; Shinozaki, 1961).

One consequence of the decrease in the inbreeding coefficient is a temporary decrease of recessive homozygotes, and if, in this situation, the mutation rate is estimated on the assumption of population equilibrium, it will be an underestimate. Once inbreeding is relaxed, the population (strictly speaking, the gene frequency) moves toward a new equilibrium, and to reach this new equilibrium several thousands of years are usually required.

Another factor which breaks down the equilibrium is change in the selection coefficient against recessive homozygotes. The progress of medical protection of genetic diseases and the improvement of environments will perhaps decrease the selection coefficient. Once the selection coefficient is changed, it again requires a large number of generations to reach a new genetic equilibrium.

The specific purposes of this paper are to examine the time required for a population to approximate a new equilibrium with a given degree of closeness after the change of inbreeding and selection coefficients, and to develop a method for estimating the mutation rate that is applicable to a population in which the equilibrium has recently been broken down.

Time Required to Approximate a New Equilibrium with a Given Degree of Closeness

Consider an equilibrium population in which the frequencies of the three genotypes for a pair of genes A and a are given as follows:

Genotype	Frequency	Adaptive value
AA	$(1 - f)p^2 + fp$	1
• Aa	$2(1 - f)pq$	1
aa	$(1 - f)q^2 + fq$	$1 - s$

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where f is the inbreeding coefficient and q the frequency of gene a ($p = 1 - q$). If the mutation rate from A to a is denoted by μ , the equilibrium condition is $(1 - f)q^2 + fq = \mu/s$, and the gene frequency is given by

$$q = \frac{-sf + \sqrt{s^2f^2 + 4\mu s(1-f)}}{2s(1-f)} \quad (1)$$

If f is changed to f' in a generation, the gene frequency begins to change gradually to reach a new equilibrium point, which is

$$q_{\infty} = \frac{-sf' + \sqrt{s^2f'^2 + 4\mu s(1-f')}}{2s(1-f')} \quad (2)$$

Theoretically, this point is reached after an infinite number of generations. If we denote the difference $q_{\infty} - q$ as Δ_0 and the difference between q_{∞} and the gene frequency in the g th generation, q_g , as Δ_g , then Δ_g is given approximately by

$$\Delta_g = \Delta_0 e^{-s[f' + 2(1-f')q_{\infty}]g} \quad (3)$$

Thus, the number of generations required for Δ to be halved is

$$g_h = \frac{0.693}{s[f' + 2(1-f')q_{\infty}]} \quad (4)$$

On the other hand, the number of generations required for Δ to be as small as 100a per cent of q_{∞} is

$$g_a = \frac{1}{\sqrt{s^2f'^2 + 4\mu s(1-f')}} \log \frac{s(f-f') + (1-f)\sqrt{s^2f'^2 + 4\mu s(1-f')} - (1-f')\sqrt{s^2f^2 + 4\mu s(1-f)}}{a(1-f)[\sqrt{s^2f'^2 + 4\mu s(1-f')} - sf']} \quad (5)$$

If g_a is multiplied by the average reproductive age, A , the number of years required for Δ to be as small as 100a per cent of q_{∞} (t_a) is obtained, i.e., $t_a = Ag_a$.

Example. Neel *et al.* (1949) estimated $q = 5 \times 10^{-3}$, $f = 6 \times 10^{-3}$, $s = 0.5$ and $\mu = 2.8 \times 10^{-5}$ for albinism in Japan. Using these data and assuming $f' = 0$, we obtain $g_h = 92.7$ and $g_{.05} = 248.9$. The present value of A in Japan is approximately 30, so that $t_h = 2,780$ years and $t_{.05} = 7,467$ years.

If both f and s are changed to f' and s' , respectively, the number of generations required for Δ to be as small as 100a per cent of q_{∞} is

$$g_a = \frac{1}{\sqrt{s^2f'^2 + 4\mu s(1-f')}} \log \frac{s'(f-f') + s(1-f)\sqrt{s'^2f'^2 + 4\mu s'(1-f')} - s'(1-f')\sqrt{s^2f^2 + 4\mu s(1-f)}}{a(1-f)[\sqrt{s'^2f'^2 + 4\mu s'(1-f')} - s'f']} \quad (6)$$

ESTIMATION OF MUTATION RATES

In the following we consider a situation in which the inbreeding and selection coefficients are changed from f and s to f' and s' respectively, in a genera-

tion, and in this generation the mutation rate is estimated. A situation close to this model is now being observed in Japan. As mentioned previously, the inbreeding coefficient is steadily decreasing at present and the improvement of environments in recent years is also remarkable, thus probably affecting the values of selection coefficients against some kinds of genetic diseases. It should, however, be noted that the formula to be developed can be applied also to some other specific situations, as will be mentioned later.

In this model the frequency of recessive homozygotes (P) in the generation in which f and s are changed is given by

$$P = (1 - f')(1 - s'_1)(q + \mu)^2 + f'(1 - s'_1)(q + \mu) \quad (7)$$

where s'_1 is the coefficient of selection exerted before the time of observation and $(1 - q)\mu$ is approximated by μ since $q\mu$ is very small. The selection coefficient after observation time is denoted by s'_2 , so that $s'_1 + s'_2 = s'$. If, for instance, observation is made at birth, s'_1 represents the prenatal selection. Since q is a function of square root μ , equation (7) is biquadratic with respect to μ and therefore it is not easy to solve in general. However, except for the case where f is unusually large, $q + \mu$ can be approximated by q , since μ is very small compared with q . Thus, neglecting μ in equation (7) and substituting for q with the relation given in equation (1), we have

$$\mu = \frac{-B + \sqrt{B^2 - 4AC}}{2A} \quad (8)$$

where

$$A = (1 - f')^2(1 - s'_1)^2$$

$$B = s(1 - s'_1)[f'(f - f')s(1 - s'_1) - 2(1 - f)(1 - f')P]$$

$$C = s^2[(1 - f)^2P - f(f - f')(1 - s'_1)]P$$

This general formula is somewhat complicated, but in the following two cases it reduces to a very simple form.

Case 1. If all types of consanguineous marriages are avoided in a genera-

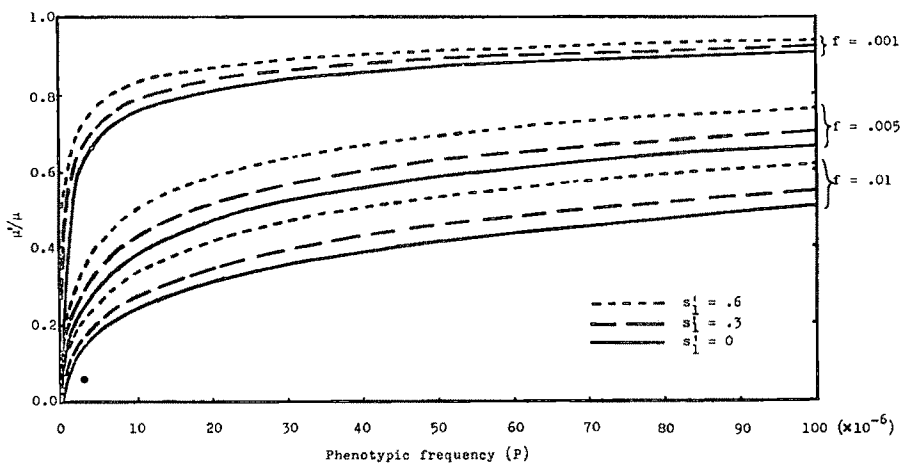


FIG. 1. Effects of f and s' on the values of μ'/μ .

tion, the inbreeding coefficient in the next generation (f') becomes 0. In this case μ is estimated by

$$\mu = \left[(1 - f) \frac{P}{1 - s'_1} + f \sqrt{\frac{P}{1 - s'_1}} \right] s \quad (9)$$

with the approximate variance of

$$\sigma^2_{\mu} = \left[\left(\frac{1 - f}{1 - s'_1} \right)^2 \frac{P(1 - P)}{N} + \frac{f^2}{1 - s'_1} \frac{1 - P}{4N} \right] s^2 \quad (10)$$

where N is the total number of observations on which P is based.

In this case, if the population is supposed incorrectly to be in equilibrium with random mating, the mutation rate is estimated to be

$$\mu' = \frac{s}{1 - s'_1} P$$

Thus, the ratio of μ' to μ is

$$\frac{\mu'}{\mu} = \frac{1}{1 + f \left(\sqrt{\frac{1 - s'_1}{P}} - 1 \right)} \quad (11)$$

The numerical relations between μ'/μ , f , s'_1 and P are given in Figure 1, and it will be seen that μ'/μ is small when f is large and s'_1 is small. For example, if $f = 0.01$, $s'_1 = 0$ and $P < 6 \times 10^{-6}$, then μ' is always less than one-fifth of the real mutation rate.

Case 2. If $f' = f$, formula (8) becomes

$$\mu = \frac{s}{1 - s'_1} P \quad (12)$$

with

$$\sigma^2_{\mu} = \left(\frac{s}{1 - s'_1} \right)^2 \frac{P(1 - P)}{N} \quad (13)$$

$$\mu = [(1 - f)q^2 + fq]s \quad (14)$$

if q is estimated by some other methods (cf. Kimura, 1958; Nei, 1963). Note that formula (14) is independent of s'_1 .

NUMERICAL EXAMPLES

The data given in the following examples are not those taken after f and s changed drastically but will suffice to illustrate the foregoing methods of estimation.

Example 1. Albinism. Tanaka and Watanabe (1960) and Tanaka (1960) found nine albinos among 197,448 school children in Shizuoka Prefecture, Japan, the phenotypic frequency being $P = 4.557 \times 10^{-5}$, while Neel *et al.* (1949) estimated the inbreeding coefficient of the past in Japan to be about 0.006 and the selection coefficient for albinism to be 0.5. The present value of the inbreeding coefficient is believed to be 0.002 approximately. According to Tanaka and Watanabe (1960), s'_1 can be assumed to be 0. Hence, the mutation rate is obtained from formula (8) and becomes

$$\mu = 3.7 \times 10^{-5}.$$

Example 2. Phenylketonuria. In a genetic study of phenylketonuria in Japan, Tanaka *et al.* (1961) estimated the segregation ratio of this disease from unaffected parents to be 0.119 ± 0.045 and ascribed its discrepancy from 0.25 to the probable high mortality of phenylketonurics. If their conclusion is correct, there must have been 59.5 per cent more deaths for phenylketonurics than for normals, that is, $s'_1 = 0.595$, since

$$s'_1 = \frac{1 - 4p}{1 - p}$$

where p is the segregation ratio. Another estimate of s'_1 is obtainable from the difference between the expected and observed frequencies of phenylketonurics, using the following formula.

$$s'_1 = 1 - \frac{\text{Observed frequency}}{\text{Expected frequency}}.$$

The expected frequency is obtained by estimating the gene frequency from the data of consanguineous marriages and computing the phenotypic frequency as expected (see Tanaka *et al.*, 1961). Using their data, this estimate of s'_1 becomes 0.553. The average of the two estimates is therefore 0.574.

On the other hand, s can be assumed to be unity. The incidence of phenylketonurics in the general population was estimated to be 16-18 per million and we take $P = 1.7 \times 10^{-5}$. As mentioned previously, the rate of consanguineous marriages in Japan is gradually decreasing at present, particularly since about 1950, but we assume $f' = f$, because almost all parents of phenylketonurics examined by Tanaka *et al.* (1961) were married before 1950. Thus, the mutation rate for this disease is estimated by formula (12) as follows:

$$\mu = \frac{s}{1 - s'_1} P = 3.99 \times 10^{-5}$$

DISCUSSION

In the derivation of the formulas given above it was assumed that f and s are suddenly changed in a generation. This assumption may be fulfilled in present Japan but probably not in Europe or in North America. According to Stern (1949), the inbreeding coefficient in France and Germany has been decreasing for about 100 years. Strictly speaking, therefore, the formulas developed here do not hold in these countries. However, as may be seen from equation (3) the amount of change in gene frequency in one generation is very small, and since 100 years correspond to only 3 or 4 generations, the amount of change in gene frequency due to decrease of inbreeding during the past century is considered to be very small. Thus, our formulas may be applicable even to the European or American populations, if the inbreeding coefficient of 100 years ago can be estimated. The same argument may hold also for the change in selection coefficient.

In this connection it should perhaps be noted that consanguineous marriages occur even under random mating and therefore they themselves are never the indication of positive inbreeding. The frequency of consanguineous marriages expected under random mating increases as the population size decreases. If, therefore, the size of population is comparatively small, say, less than 1,000,

the chance consanguineous marriages should be subtracted from the total frequency of consanguineous marriages in estimating the inbreeding coefficient (Nei and Imaizumi, unpublished).

SUMMARY

The mutation rate in rare recessive traits of man is usually estimated on the assumption that the population under investigation is in equilibrium. This assumption, however, is not necessarily fulfilled because of changes in inbreeding and selection coefficients in recent years. In view of this situation a refined method is proposed for estimating the mutation rate, removing the assumption of population equilibrium. A formula giving the time required for a population to reach a new equilibrium after change of inbreeding and/or selection coefficient with a certain degree of closeness is also presented.

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Studies on Hereditary Gamma Globulin Factors: Evidence that Gm (b) in Whites and Negroes is not the Same and that Gm-like is Determined by an Allele at the Gm Locus

ARTHUR G. STEINBERG AND JANET A. WILSON

*Department of Biology,
Western Reserve University, Cleveland, Ohio*

TWO GENETICALLY INDEPENDENT loci determine, through a number of co-dominant alleles, a series of gamma globulin factors detected by an agglutination-inhibition test. The Gm locus has been shown to determine factors Gm (a), Gm (b), Gm (x), Gm (r), a "silent factor" which does not react with any known reagent [see Steinberg, 1962, for review], and perhaps Gm (d) [Thomas and Kampf, 1961] and Gm (e) [Ropartz, Rivat and Rousseau, 1962]. The Inv locus determines factors Inv (a), Inv (b), a "silent" factor (Steinberg, 1962) and Inv (1) [Ropartz, personal communication]. Another gamma globulin factor, Gm-like, has, with one exception (Steinberg, Stauffer, and Dunsford, in preparation), been found in Negroes only.

Because the γ -globulin factors in serum from Negroes of unmixed ancestry tested with the standard reagents have all been Gm (a+ b+ x—) [the reagents for detecting Gm (r) and Gm (d) are not available, hence data for these factors and the newly found Gm (e) were not collected] it was not possible to determine the genetic relation of Gm-like to the Gm locus. Attempts to find segregation at the Gm locus and for Gm-like in American Negro families have thus far been unsuccessful.

Ropartz and his colleagues (Ropartz, Rousseau, Rivat, and Lenoir, 1961) using the factor Inv (a) demonstrated that Gm-like is independent of the Inv locus and we have confirmed their findings using the Inv (b) factor (Steinberg, Wilson, and Lanset, 1962).

We have recently found a system which appears to distinguish between the Gm (b) factor of whites and Negroes. This paper is a report of the data establishing the nature of the reactions with this system and of its use to ascertain the locus which determines Gm-like.

MATERIALS AND METHODS

The γ -globulin factors were detected by methods which we have described elsewhere (Steinberg, 1962) except that some of the tests were run in tubes as described by Linn-Jepsen, Galatius-Jensen and Hauge (1958). The reagents used are shown in table 1.

The new agglutinator was discovered in May 1962, in the serum drawn in 1959 from

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a four year old Negro boy in the course of determining the γ -globulin factors in his serum and in the serum of members of his family. The data were to be used for a linkage study with the sickle locus (these data will be reported elsewhere). It was found that the boy's serum agglutinated cells coated with incomplete anti-D Roehm which we use to detect Inv (a). Small samples of blood were drawn on May 8, June 7, and July 26, 1962. These samples were used to characterize the system, Davis/Roehm, and for family tests.

The system, Davis/Roehm, works best when used as follows: One volume of group O, R¹R¹ washed, packed red blood cells is incubated with two volumes of anti-D Roehm plus five volumes of saline at 37° C. for two hours. The cells are washed and made into a 1.5 per cent suspension in saline.

The serum to be tested is diluted 1/16. The Davis serum is diluted 1/4. One drop of each is placed in a 10 x 75 mm. test tube and incubated for 15 minutes at room temperature, then one drop of the above cell suspension is added. Incubation is continued at room temperature for another 90 minutes. The tubes are spun at 1,000 G for one minute. The tests are read immediately after spinning, in the usual manner.

RESULTS

Tests of sera from white donors: Samples of serum from 99 white donors previously tested for the γ -globulin factors were tested with the Davis/Roehm system. The tests agreed with previous typings for the Gm (b) factor: 66 were Gm (b+) and 33 Gm (b-) on both series of tests.

Tests of sera from Negro donors: Serum from 291 unrelated Negroes whose γ -globulin types were known were tested with the Davis/Roehm system. The results of these tests are shown in table 2. For simplicity of presentation Gm-like (+) is recorded as c.

TABLE 1. REAGENTS USED FOR γ -GLOBULIN TYPING

System	Agglutinator	Anti-D	Dilution of Test Serum
Gm (a)	Wils 1/8	Kim 1/10	1/8, 1/16
Gm (b)	Bomb 1/64	Ivan 1/10	1/8, 1/16
Gm (x)	Taylor 1/32	Ham 1/10	1/16, 1/32
Gm (like)	Carp 1/32	Warren 1/5	1/16, 1/32
Inv(a)	Math 1/8	Roehm 1/5	1/16, 1/32
¹ Inv(b)	Lucas 1/8	Ham 1/5	1/16, 1/32

¹This is a tube test.

TABLE 2. RESULTS OF TESTS OF SERUM FROM 291 UNRELATED NEGROES (105 FROM A NON-RANDOM PANEL AND 186 RANDOM SAMPLES) WITH AGGLUTINATOR DAVIS AND ANTI-D ROEHM. NUMBERS IN PARENTHESES ARE DATA FROM THE RANDOM SAMPLE

	Gm Phenotypes	Total	Davis/Roehm
(1)	Gm [a+ b+ x- (c*+ or -)]	256(172)	55 ⁺ (35) [†] 201(137) [‡]
(2)	Gm [a+ b+ x+ c-]	9(6)	3(2) 6(4)
(3)	Gm [a- b+ x- c-]	8(4)	8(4) 0(0)
(4)	Gm [a+ b+ x+ c+]	4(0)	0(0) 4(0)
(5)	Gm [a+ b- x- c-]	11(3)	0(0) 11(3)
(6)	Gm [a+ b- x+ c-]	3(1)	0(0) 3(1)
	Totals	291(186)	66(41) 225(145)

*c = Gm-like

[†]8 of these 35 were Gm-like (+).

[‡]46 of these 137 were Gm-like (+) and 6 for various reasons were not classified for Gm-like.

None of the 14 samples which were G_M (b—) were Davis/Roehm (+). Similarly none of the four G_M (a+ b+ x+ c+) samples were Davis/Roehm (+). Each of the other groups showed (+) and (—) reactions with the Davis/Roehm system. Since we had established that this system tested for G_M (b) in whites we assumed, as a working hypothesis, that this system failed to detect the G_M (b) factor determined by the *Gm^{ab}* allele in Negroes (Steinberg, Stauffer and Boyer, 1960). The positive reactions among the samples from Negroes were assumed to be due to the *Gm^b* allele derived from white ancestors. Population and family data may be used to test this hypothesis.

Steinberg *et al.* (1960) showed that Negroes of unmixed ancestry have a *Gm^{ab}* allele and that the *Gm^b* allele derived from white ancestry occurs in the American Negro with a frequency (q) of $.160 \pm .020$. Hence, $[1 - (.84)^2] \pm 2p\sigma_p = 29.4 \pm 3.4$ per cent of a random sample of sera from American Negroes should be Davis/Roehm (+). From the data in parenthesis in table 2 it will be seen that 41/186 or 22.0 ± 3.0 per cent were positive. The difference is not significant ($D/\sigma_D = 1.6$, $P = .11$).

Family studies: The sera from 47 American Negro families with 174 children were typed for G_M (a), G_M (b), G_M (x), G_M-like and for their reaction with the Davis/Roehm system. The data are presented in table 3. Davis/Roehm (+) is recorded as D in the table.

All of the 70 offspring from matings in which neither parent was D (matings 1-4) were Davis/Roehm (—). This is what would be expected if D were determined by a dominant gene. If we ignore G_M-like for the present, the genotypes of the parents in matings 1-3 may be assumed to be *Gm^{ab}/Gm^{ab}* and those for the parents in mating (4) *Gm^{ab}/Gm^a* X *Gm^a/Gm^a* since G_M (a+ b—) offspring appeared in each of the two matings of this type.

Consider now, matings 5-12 in which only one parent is D. We will dis-

TABLE 3. RESULTS OF TESTING SERA FROM 47 FAMILIES WITH 174 CHILDREN FOR G_M (A), G_M (B), G_M (X), G_M-LIKE AND FOR REACTIONS WITH THE DAVIS/ROEHM SYSTEM. ONLY POSITIVE REACTIONS ARE LISTED (G_M-LIKE (+) IS RECORDED AS C AND DAVIS/ROEHM (+) AS D.)

Mating	No. of Families	No. of Offspring	Phenotypes of Offspring							
			ab	abc	abD	abcD	bD	a	ax	abxD
(1) ab X ab	3	18	18							
(2) ab X abc	5	31	14	17						
(3) abc X abc	4	13	7	6						
(4) ab X a	2	8	2					6		
(5) ab X abD	10	25	12		13					
(6) ax X abD	1	2			1					1
(7) ab X bD	1	6	1		5					
(8) a X abD	1	1			1					
(9) abx X abD	1	9	2		3				4	
(10) ab X abcd	6	27		17	10					
(11) abc X abD	4	10	3	5		2				
(12) abc X abcd	2	4		2	1	1				
(13) bD X abD	3	11			7		4			
(14) abD X abD	2	6			5		1			
(15) abD X abxD	1	2					2			
(16) abcd X abD	1	1			1					
Totals	47	174	59	47	47	3	7	6	4	1

cuss these matings in turn except for mating 7 which will be discussed with mating 13 after all the other matings have been reviewed. As before we will ignore Gm-like. In all of these matings (except 7) the phenotype of the Davis/Roehm (+) parent is abD. On the basis of our hypothesis the genotype of each of these parents is either Gm^{ab}/Gm^b or Gm^a/Gm^b . In either case the parents is heterozygous for the Gm^b allele (i. e., for D). Hence the ratio of D to non-D among the offspring should be 1:1. The ratio of D: non-D among the 78 offspring from these matings was 33: 45, which is not significantly different from 39: 39 ($\chi^2_{(1)} = 1.85$, $P > .10$).

Consider now, matings 14-16 in which both parents have the D factor. We will again ignore Gm-like (c in the table). In each of these matings the parents' phenotypes are abD or abxD, their genotypes are, according to our hypothesis, Gm^{ab}/Gm^b or Gm^a/Gm^b for the former, and Gm^{ax}/Gm^b for the latter. In any case both parents are heterozygous for Gm^b and therefore for D. We would expect 1/4 of the nine offspring from these families to be non-D, but all nine were D. The probability of all nine offspring being D with $p = .75$ is approximately .075, hence the failure to observe non-D offspring is not significant ($\chi^2_{(1)}$, corrected for continuity = 1.81; $P > .10$; this is a two-tailed distribution).

If we combine the data from the two types of matings and compare the totals with the combined expected we have: observed 42: 45, expected 45.75: 41.25 of D vs. non-D. The differences are not significant ($\chi^2 = 0.648$, $.5 > P > .3$).

In the remaining two families (7 and 13) one parent is phenotypically bD. According to our hypothesis the corresponding genotype is Gm^b/Gm^b . Since the parent is homozygous for the Gm^b allele all the offspring from these matings should be D, but one child in mating 7 is non-D. In this mating the Gm^b/Gm^b parent was the father, hence the possibility exists that the non-D child is extra-marital, however, no exclusion was found on the basis of the ABO, MNS, Rh, Fy, K, P, Jk and Js blood types, and the Hp and Tf serum factors.

Sera from West African Negroes: Samples from 28 Yoruba and 27 Fulani were tested with the Davis/Roehm system. Data on their Gm types have been published (Steinberg, Stauffer, Blumberg, and Fudenberg, 1961).

According to our hypothesis all these samples should be negative for the Davis/Roehm system. Fifty-four of the 55 samples were negative. One serum from a Yoruba was positive with the Davis/Roehm system each of the three times it was tested. The Gm type of this sample was Gm (a+ b+ x—), Gm-like (+), in accordance with the usual pattern of the African Negro. If the donor was of unmixed ancestry, and he most probably was so, this is a distinct exception to our hypothesis and indicates that occasionally a variant of the Gm^{ab} allele which produces a Gm (b) factor positive to the Davis/Roehm system may occur.

Sera from Chinese: Chinese have the alleles Gm^a , Gm^{ab} and Gm^{ax} (Steinberg, Lai, Vos, Singh, and Lim, 1961), hence it seemed of interest to determine the reaction of the Gm (b) factor in Chinese with the Davis/Roehm system. Forty-seven samples collected by Dr. David Y.-Y. Hsia in Formosa

were tested with the results shown in table 4. All G^m (b+) samples were positive with the Davis/Roehm system and all G^m (b-) samples were negative with the Davis/Roehm system. Evidently the G^m (b) factor produced by the G^{m^{ab}} allele in Chinese is similar to that produced by the G^{m^b} allele in whites rather than to that produced by the G^{m^{ab}} allele in Negroes.

Relation of G^m-like to the G^m locus: In table 3 G^m-like is represented by the letter c and positive reactions with the Davis/Roehm system are represented by D. In matings 10, 11, 12, and 16 one or both parents have factors c and D and offer possibilities for studying segregation between the alleles determining G^m-like and G^m (b) (i. e., G^{m^b}). The data for these matings are presented again with additional information in table 5. Most information is provided by mating 10 (ab X abcD). With c and D present in one parent and absent in the other, none of the 27 offspring showed both factors or neither factor. This would be expected if G^m-like were due to an allele at the G^m locus, or to a closely linked gene, with the cD parent in repulsion in all six matings of this type. Since G^m-like appears not to occur in the absence of G^m (b) (Steinberg, 1962) and since earlier evidence suggested a relation between G^m-like and G^m (b) (Steinberg, Stauffer, Blumberg and Fudenberg, 1961), we will assume that G^m-like is produced by an allele at the G^m locus and call the factor G^m (c). We have already indicated our belief that in whites D is produced by the G^{m^b} allele. The genotypes of the parents in mating 10 are therefore G^{m^{ab}}/G^{m^{ab}} or G^{m^{ab}}/G^{m^a} for phenotype ab and G^{m^{abc}}/G^{m^b} for phenotype abcD (table 5). Hence the offspring would inherit G^{m^{abc}} or G^{m^b} from the latter parent and none should have neither or both of factors c and D. Furthermore, the two types of offspring should occur with equal frequency. The ratio of 17: 10 does not differ significantly from 13.5: 13.5 ($\chi^2_{.1} = 1.81$, $P > .1$). The genotypes of the parents of mating 10, already referred to, and of matings 11, 12, and 16 and the frequencies of the phenotypes among their offspring expected as a consequence of this hypothesis are presented in table 5. The hypothesis appears to be consistent with the data.

If G^m-like is caused by an allele at the G^m locus, the four phenotypic classes, c+ D+, c+ D-, c- D+, c- D-, in the random population sample in table 2 should occur in accordance with the Hardy-Weinberg distribution.

The frequency of the G^{m^b} allele, hereafter referred to as D, may be estimated by direct count. It is equal to

$$p = \frac{abcD + abD + abxD + 2bD}{2T}, \text{ (Where } abcD, \text{ etc.,}$$

TABLE 4. TESTS WITH THE DAVIS/ROEHM SYSTEM OF SERUM FROM 47 MAINLAND CHINESE LIVING IN FORMOSA

G ^m phenotype	No.	Reaction with	
		+	-
• ab	31	31	0
abx	8	8	0
ax	2	0	2
a	6	0	6
Totals	47	39	8

TABLE 5. GENOTYPES OF MATINGS 10, 11, 12 AND 16 OF TABLE 3, AND OBSERVED AND EXPECTED NUMBERS OF PHENOTYPES AMONG THEIR OFFSPRING ON THE ASSUMPTIONS THAT Gm-LIKE IS PRODUCED BY AN ALLELE Gm^{abc} AND THAT Gm^b PRODUCES THE FACTOR WHICH IS POSITIVE WITH THE DAVIS/ROEHM SYSTEM

Mating	Phenotype	Most likely genotypes	Phenotypes of Offspring for c and D							
			c		D		cD		neither	
			Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
10	ab X abcD	Gm^{ab}/Gm^{ab} X Gm^{abc}/Gm^b	17	13.5	10	13.5				
11	abc X abd	Gm^{abc}/Gm^{ab} X Gm^{ab}/Gm^b	5	2.5	0	2.5	2	2.5	3	2.5
12	abc X abcD	Gm^{abc}/Gm^{ab} X Gm^{abc}/Gm^b	2	2.0	1	1.0	1	1.0		
16	abcD X abd	Gm^{ab}/Gm^b X Gm^{ab}/Gm^b	0	0.25	1	0.5	0	0.25		
			24	18.25	12	17.5	3	3.75	3	2.5

$\chi^2_s = 3.786, .3 > P > .2$

are the phenotypes and T equals the total sample.).

Hence,

$$p = \frac{8 + 27 + 2 + 8}{360} = .1250.$$

The frequency of the G_m^{abc} allele, referred to as c, may be estimated as follows:

$$\begin{aligned} q &= 1 - \frac{\sqrt{ab + abD + abx + abxD + bD + a + ax}}{T} \\ &= 1 - \frac{\sqrt{85 + 27 + 4 + 2 + 4 + 3 + 1}}{180} \\ &= 1 - \sqrt{.700} = .1633. \end{aligned}$$

Finally, the combined frequency of the alleles which do not lead to either c or D = μ = 1 - p - q = .7117. The phenotypes may be represented as c+ D+, c+ D-, c- D+, and c- D- by ignoring the factors a, b, and x. When this is done the observed and expected frequencies (the latter computed on the Hardy-Weinberg equilibrium) of the four classes are as shown in the first and second rows of table 6. The agreement between the observed and the expected frequencies is satisfactory.

Ropartz *et al.* (1962) used the factor G_m (e), which is determined by an allele at the G_m locus and which segregates in Negroes, to test whether G_m-like is determined by an allele at the G_m locus or by one at a separate locus. As is well known, this test consists of determining whether the four phenotypes (in this case c+ e+, c+ e-, c- e+, and c- e-) resulting from the two factors may be assumed to be due to random distribution of the factors. If the answer is yes, the assumption is that two loci are involved. Ropartz's analysis showed that c (G_m-like) and e were randomly distributed with respect to each other and he concluded, therefore, that G_m-like is due to an allele at a different locus from the G_m locus. Our data, analysed in the same manner (table 6, rows one and three), also may be assumed to show independent assortment of c and D, *i. e.*, to show that c is due to an allele at a locus other than the G_m locus. The point is simply that the population sample is not sufficiently large to distinguish between the alternative hypotheses.

Unfortunately, it is customary for investigators to apply only one of the

TABLE 6. COMPARISON OF OBSERVED AND EXPECTED FREQUENCIES OF c+ D+, c+ D-, c- D+, AND c- D- PHENOTYPES (SEE TEXT)

	c+D+	c+D-	c-D+	c-D-	Total
Observed	8	46	33	93	180
Expected ^a	7.3	46.7	34.8	91.2	180.0
Expected ^b	12.3	41.7	28.7	97.3	180.0
•	(a) $\chi^2_{(1)}$	= 0.21		.7 > P > .5	
	(b) $\chi^2_{(1)}$	= 2.79		.10 > P > .05	

^aComputed on the basis of the Hardy-Weinberg equilibrium.

^bComputed on the assumption of random distribution of alleles at two loci.

above tests to their data if the analysis shows a satisfactory fit. The analyses of our data show that it would be wise to test population data by both methods before drawing conclusions.

For the reasons stated earlier, we consider Gm-like to be due to an allele at the Gm locus. Ideally, we would test a larger population sample to exclude independent distribution of the c and 'D' factors. Since the donor is a child it is difficult to obtain sufficient reagents to do this in a reasonable period of time; accordingly we have decided to present the data as they are.

DISCUSSION

The donor of the agglutinating serum is a healthy Negro boy whose serum is negative for rheumatoid factor activity as measured by the latex test (Singer, Altmann, Goldenberg, and Plotz, 1960) and by the modified Waaler-Rose test (Podliachouk, Eyquem, and Jacqueline, 1958). The agglutinating activity was first identified in a sample drawn when the child was only four years old. It was still present in samples drawn three years later. None of the other members of his family has serum which agglutinates red blood cells coated with the anti-D sera which we use routinely in our laboratory. It will be of interest to study the serum and the clinical status of this boy during the course of the next several years.

The Gm and Inv types, as determined by our usual reagents (table 1), of the donor and the members of his family and the reactions of their sera with the Davis/Roehm system are shown in table 7. It is of interest that the donor's genotype is probably Gm^{ab}/Gm^{ab} . This supports our contention that the Gm (b) produced by the Gm^{ab} allele is different from the Gm (b) produced by the Gm^b allele. Healthy donors of agglutinating sera have invariably been negative for the factor which their sera detect.

The distinction between the Gm (b) factor of whites and Negroes was foreshadowed by Ropartz's observation (personal communication), confirmed by us, that sera from some Gm (b+) Negro donors were negative with SNagg Letendre which tests for Gm (b) in the serum of white donors. We have other agglutinators which also fail to detect the Gm (b) factor in the serum of some Gm (b+) Negroes but not in all. It is apparent that the Gm (b) factor in Negroes, at least, is very variable. This is similar to the variation in Negroes of the D factor of the Rh locus (Race and Sanger, 1962).

The observation that the Gm (b) factor produced by the Gm^{ab} allele in Chinese is similar to the Gm (b) factor in whites indicates that the distinctness

TABLE 7. Gm AND INV TYPES OF THE DAVIS FAMILY AS DETERMINED BY STANDARD REAGENTS (TABLE 1) AND REACTIONS OF THEIR SERA TO DAVIS/ROEHM SYSTEM (ONLY POSITIVE REACTIONS ARE SHOWN)

	Gm	Inv	Davis/Roehm	Probable Gm Genotype
Fa	ab	b	—	Gm^{ab}/Gm^{ab}
Mo	ab	ab	+	Gm^{ab}/Gm^b
1*	ab	b	—	Gm^{ab}/Gm^{ab}
2	ab	ab	—	Gm^{ab}/Gm^{ab}
3	ab	b	+	Gm^{ab}/Gm^b

*Donor.

of G_M (b) in Negroes is not due to its production by an allele which also produces G_M (a).

The two exceptions to our hypothesis (a child who was negative to Davis/Roehm when he should have been positive, and an African Negro positive to Davis/Roehm when he should have been negative) are disturbing. The exceptional child could be due to extra-marital origin, but it seems unlikely that the African Negro donor had mixed ancestry. Although sera from several populations were sent from Dr. Blumberg's laboratory at the same time, it is unlikely that this serum was from a non-Negro donor because it is G_M (c+). It is possible that the reaction of this serum represents a mutation from the G_M^{ab} allele found in Negroes to the G_M^{ab} allele found in Chinese or, less likely, to the G_M^b allele found in whites. Clearly, further data are required.

We suggest that G_M (b) factors detected by the Davis/Roehm system be called G_M (bw) and that the alleles determining these factors be indicated as G_M^{bw}, G_M^{abw}, etc.

The family data, although not as extensive as one would desire, indicate that G_M-like is due to an allele at the G_M locus. This conclusion is bolstered by the observation that G_M-like has been observed only when the factors G_M (a) and G_M (b) were also present. The failure of our relatively small population sample to exclude the hypothesis of two loci does not appear to be of great import. The fit to the Hardy-Weinberg distribution is also satisfactory and indeed somewhat better than that obtained from a comparison with a random distribution.

If our assumption that G_M-like is due to an allele at the G_M locus is correct, the G_M factor should be found if at all on the B fraction resulting from the digestion of gamma globulin with papain, and not in the A-C fraction (Franklin, Fudenberg, Meltzer, and Stanworth, 1962; Harboe, Osterland, and Kunkel, 1962). Furthermore, it should not be present in Bence-Jones proteins or in β_{2M} or β_{2A} globulins which do not appear to have a fraction corresponding to the B fraction.

SUMMARY

A serum from a four year old healthy Negro boy (Davis) was found to cause red blood cells coated with an incomplete anti-D serum (anti-D Roehm) to agglutinate. The serum had been tested three years after it was drawn. Further tests showed that this system (Davis/Roehm) detected the G_M (b) factor determined by the G_M^b allele in whites and the G_M^{ab} allele in Chinese but not by the G_M^{ab} allele in Negroes. The Davis/Roehm system has enabled us to demonstrate that G_M-like is determined by an allele at the G_M-locus or by a gene closely linked to this locus. On the basis of earlier studies we have concluded that the former is more likely. Accordingly, we have decided to refer to G_M-like as G_M (c). Negroes appear to have an allele G_M^{abc} which produces factors G_M (a), G_M (b), and G_M (c).

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Sex Linkage, Inbreeding, and Growth in Childhood

WILLIAM J. SCHULL AND JAMES V. NEEL

*Department of Human Genetics, University of Michigan
Medical School, Ann Arbor, Michigan*

MARRIAGES WHICH INVOLVE INDIVIDUAL SUBJECTS biologically related to one another are subdivisible, first, with respect to the number of individuals interposed between the spouses and their common ancestor(s), that is, by the degree of relatedness, and second, within a given degree, by the sex of the individuals through whom descent passes. Consider, for example, marriages which involve the offspring of siblings, that is, marriages of first cousins. When the sex of the individuals through whom descent passes is considered, four sub-types of this marriage can be recognized. They are the marriage of the offspring of brothers, of sisters, of a brother's son with a sister's daughter, or of a brother's daughter with his sister's son. Other consanguineous marriages, such as first cousins once removed or second cousins, give rise to more numerous sub-types. An extended account and enumeration of many of the more common human relationships is to be found in a recent paper by Haldane and Jayakar (1962).

For genes located on the autosomal chromosomes, clearly the sub-type of the consanguineous marriage is of little importance, but this is not so with respect to genes borne on the X-chromosome. Consider, again, the marriage of first cousins. For sex-linked genes, sons born to any one of the four possible sub-types are, of course, hemizygous. Daughters, on the other hand, may be either homozygous or heterozygous at a given X-linked locus. If they are homozygous, they may be so by virtue of genes (a) alike in function and identical in origin, that is, descended from a single gene present in an ancestor, or (b) alike in function but not in origin. The probability of the former event, termed the coefficient of inbreeding, is easily computed for sex-linked genes (see Haldane and Moshinsky, 1939). It is not difficult to show that this probability, say F' , is zero for daughters born to spouses who are the offspring of brothers or are related as a brother's son and a sister's daughter. This probability is $3/16$, however, for the daughters of first cousins related through two sisters, and $2/16$ for daughters born to a sister's son and her brother's daughter. To obtain these values we merely compute F' as for an autosomal locus, but omit any male in the path and any path with two successive males (Wright, 1951; see page 341). These differences in F' can be made the basis for evaluating, in at least a preliminary fashion, the contribution of sex-linked genes to the occurrence of a given trait. Suppose the characteristic in question was polygenic in origin. If a disproportionate fraction of these genes, or if one or more "major" modifiers associated with the polygenic system, is located on the X-chromosome, then, under certain circumstances, we may expect either the mean of the charac-

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teristic, the variance, or both to be a function of the probabilities previously given. The purpose of this paper is to report the results of applying this argument to ten anthropometric characteristics of children. It is worth noting in passing that the argument outlined is relatively more powerful for sex-linked genes than for genes on the average autosome because the range of F' is greater than that of F .

The sample: In the years 1948-1953, as part of a comprehensive attempt to assess the effect of exposure to the atomic bombs on pregnancy terminations, some 4,598 children were examined in Hiroshima or Nagasaki, Japan, who were born to parents who reported a consanguineous marriage at the time of pregnancy registration. The findings in these children at birth, and, in a subsample, at nine months of age, have already been reported (Schull, 1958; Morton, 1958). These findings were sufficiently provocative to indicate the need for further studies. As a consequence, between 1958 and 1960 follow-up studies were undertaken on these children and a suitable control group. The details of this study, known as the Child Health Survey, have been presented elsewhere (Schull and Neel, 1962). In all, 9,382 children were selected for re-examination; children whose parents received appreciable amounts of radiation were excluded from consideration. Of these children, 6,823 were still alive and residing in one or the other of the cities in the years 1958-1960. We here report the results of anthropometric examinations on 853 of these children—girls whose parents are known to be first cousins.

The data and their analysis: For each of the aforementioned 853 girls there exists an observation which consists of two concomitant variables, age and F' , and ten measurements. The latter are:

weight	head length
height	head breadth
head girth	head height
chest girth	sitting height
calf girth	knee height

All measurements were made in a clinical environment on children not acutely ill. Specially trained personnel obtained the anthropometrics in a manner consistent with Martin's definitions (1928).

Analysis of these data proceeded along two lines. First, the dependence of the measurements in question upon age and F' was assessed by means of a k -variate linear regression model. Second, an effort was made to simplify the interpretation of the data by relating to age and F' certain linear combinations of the measurements. We turn, now, to a brief consideration of the results of these two methods of analysis.

The k -variate linear model which we fitted asserts that the expected values for the j^{th} child in the i^{th} city are a linear function of age, A , and inbreeding coefficient, F' , that is, that

$$\underline{Y}_{ij} = \underline{m}_i + \underline{a}_i A_{ij} + \underline{b}_i F'_{ij} + \underline{e}_i \quad \circ$$

where $\underline{Y}'_{ij} = (Y_{1ij}, Y_{2ij}, \dots, Y_{10ij})$ is the vector of expected values, $\underline{e}'_i = (e_{1i}, e_{2i}, \dots, e_{10i})$ are the error terms, $\underline{m}'_i = (m_{1i}, m_{2i}, \dots, m_{10i})$, $\underline{a}'_i = (a_{1i}, a_{2i}, \dots, a_{10i})$, and $\underline{b}'_i = (b_{1i}, b_{2i}, \dots, b_{10i})$ are the vectors of the general mean, the age effect, and the inbreeding effect, respectively.

In the present case, we were interested in several hypotheses with regard to the \underline{a}_i and \underline{b}_i vectors. First, we wished to know whether the regressions on age and F' were common for the two cities. As shown in tables 2 and 3, they are. Thus, we may take as the "best" estimates of the vectors \underline{a} and \underline{b} the estimates derived from the pooled city data. Secondly, we were interested in knowing whether a specific vector, say \underline{a}_1 , was significantly different from a vector, \underline{Q} , whose elements are all zero. The test statistic which was used is the Mahalanobis distance criterion which in this case is

$$D^2 = [\underline{a}_1]' \Sigma^{-1} [\underline{a}_1]$$

where Σ^{-1} is the inverse of the variance-covariance matrix of the a 's, the estimates of the regression coefficients with respect to age. D^2 can be shown to be asymptotically distributed as χ^2 with degrees of freedom equal to the rank of the variance-covariance matrix (Anderson, 1958). A more measured presentation of the tests used as well as the computations which are involved will be found in Schull and Kudo (1961).

We give in table 1 the mean values of the ten variables predicted for ten-year-old daughters of first cousins whose F' is zero. These values, which reveal Nagasaki children to be somewhat different from their Hiroshima counterparts, compare satisfactorily with those published for ten-year-old females in the Gakkō Eisei Tōkei Hōkokusho (School Health Statistics, 1956, published by the Japanese Ministry of Education). The dependence of the various measures on age is illustrated in table 2 where we give the vectors of regression coefficients of the variables on age for the cities separately as well as pooled. The χ^2 associated with the test of significance of the difference between the age vector based upon the pooled data and a zero vector is 1,502.28, and the degrees of freedom are ten.

We turn now to matters of greater genetic interest, namely, to the increments and decrements of change as related to inbreeding (see table 3). Inspection of the two city vectors suggests that neither city vector nor the pooled vector differs significantly from a zero vector. This impression is supported by tests of significance; thus, a test of the b vector based upon the pooled city observations

TABLE 1. MEAN VALUES FOR CERTAIN CONTINUOUS VARIABLES PREDICTED FOR TEN-YEAR-OLD CHILDREN, GIRLS, WHOSE PARENTS ARE FIRST COUSINS BUT WHOSE COEFFICIENT OF INBREEDING FOR SEX-LINKED GENES IS ZERO

Variable	Hiroshima	Nagasaki
Weight (hectograms)	261.16	253.86
Height (mm.)	1,280.17	1,282.46
Head girth (mm.)	507.63	507.83
Chest girth (mm.)	607.27	594.16
Calf girth (mm.)	260.04	250.87
Head length (mm.)	168.55	166.95
• Head breadth (mm.)	143.43	145.25
Head height (mm.)	122.75	122.22
Sitting height (mm.)	713.66	710.10
Knee height (mm.)	326.60	324.50
No. of observations	(321)	(532)

fails to establish a significant effect of F' on the measurements ($\chi^2 = 13.97$; $df = 10$). The absence of a significant association of these measurements with F' can be interpreted either as an indication that the traits in question are not influenced by "major" sex-linked genes, or, if sex-linked genes are involved, then the heterozygotes must not differ from the average of the homozygous classes. Were the latter not so, the means should be displaced with inbreeding (see Kempthorne, 1957). Can one differentiate between these alternatives? Theoretically, yes. If the former alternative is true, the variances would be independent of F' ; whereas if the second of the alternatives is true, we would expect the variances to change with changing F' . In practice, a distinction along these lines is difficult to make, however, because of the difficulties in relating the variance-covariance matrices to F' , and, moreover, even when $F' = 1$, the variance is only doubled. If, in the present instance, one computes for each consanguinity class the determinant of the covariance matrix, after the removal of the effects of age, and compares these determinants with F' , one observes (see table 4) that:

(1) in both cities, the determinants, that is, the generalized variances, diminish with increasing F' . Moreover, if the equality of these variances is tested using the generalized form of Bartlett's criterion (Box, 1953), then

(2) the differences between the six generalized variances are significant; however,

(3) the significance seems to rest principally on the Nagasaki, $F' = O$ group, for if this group is removed, the remaining five do not differ significant-

TABLE 2. THE INCREMENT OR DECREMENT OF CHANGE PER MONTH OF AGE FOR CERTAIN CONTINUOUS VARIABLES MEASURED IN CHILDHOOD

Variable	Hiroshima	Nagasaki	Pooled
Weight	1.678	1.581	1.619
Height	4.122	4.106	4.109
Head girth	0.283	0.253	0.265
Chest girth	1.379	1.312	1.338
Calf girth	0.686	0.650	0.665
Head length	0.099	0.101	0.100
Head breadth	0.030	0.044	0.038
Head height	0.017	0.022	0.020
Sitting height	1.965	1.895	1.921
Knee height	1.273	1.272	1.272

TABLE 3. THE INCREMENT OR DECREMENT OF CHANGE PER PER CENT F' FOR CERTAIN CONTINUOUS VARIABLES MEASURED IN CHILDHOOD

Variable	Hiroshima	Nagasaki	Pooled
Weight	-0.0612	-0.2264	-0.1693
Height	0.4164*	-0.4903	-0.1681*
Head girth	0.0613	0.0576	0.0584
Chest girth	0.0840	-0.1818	-0.0884
Calf girth	-0.0358	-0.0305	-0.0330
Head length	0.0066	0.0131	0.0108
Head breadth	0.0579	0.0307	0.0406
Head height	0.0151	-0.0007	0.0050
Sitting height	0.2071	-0.2939	-0.1169
Knee height	0.1385	-0.3340	-0.1660

*Significantly different from zero at the 5 per cent probability level.

ly. The variance in question is, furthermore, conspicuously larger than the next largest variance.

If one interprets these observations as evidence that the generalized variances are not different, then mean and variance data are not contradictory. If, however, one assumes that the variances are, in fact, dissimilar, then a meaningful interpretation of the variance data becomes nigh impossible. The variance may increase or decrease as a function of dominance and different gene frequencies. At present, experience provides no rationale for any one specific model. Accordingly, we find more appealing the interpretation that the data do not support the hypothesis of sex-linked genes playing a major role in the traits under study.

Are these findings in accord with those from the second approach to the data? The latter, it will be recalled, involved the use of principal components. An exposition of the general theory underlying the use and estimation of principal components obviously lies outside the objectives of this paper. Briefly, however, it may be said that the derivation of principal components is a statistical technique for reducing a large number of correlated variables to a smaller number of uncorrelated variables. A justification of the use of principal components in the present case might proceed somewhat as follows: Our concern is primarily with the variation which exists from individual to individual and how this relates to inbreeding. If we can show that most of this variation resides in two or three hypothetical variables, linear combinations of the anthropometric measurements, then we can direct our attention to these variables rather than the observable traits. If these hypothetical variables in addition to being linear possess certain, special variance properties, they are termed principal components. It can be shown, for example, that the first principal component is the normalized linear combination of the measurements having the largest variance. It can be further shown that the principal components are the characteristic or eigen vectors of the covariance matrix, or the correlation matrix if the unit of measurement varies from one observable trait to the next, as in our case. For further information on principal components the reader is referred to Anderson (1958).

In table 5 are given the first eight principal components appropriate to the data at hand. We note that collectively they account for some 98.5 per cent of the variation between individuals in the sample. Of greater interest is the

TABLE 4. THE GENERALIZED VARIANCES AS A FUNCTION OF CITY AND F'

City	F'	n_k	$\log S_{ijk} $
Hiroshima	0	112	45.485083
	.1250	105	44.540266
	.1875	98	44.283160
Nagasaki	0	210	49.591902
	.1250	177	45.494327
	.1875	139	45.230325

n_k = degrees of freedom

F' = sex-linked coefficient of inbreeding

Homogeneity of variances (all groups)

Homogeneity of variances (exclusive of Nagasaki — 0)

$\chi^2 = 1705.31$ $df = 275$

$\chi^2 = 62.99$ $df = 220$

fact that the first three components *alone* account for somewhat more than 75 per cent of the total variation. Thus, if we restrict our attention to just the first three components and their behavior with respect to age and F' , we are, in effect, studying the bulk of the variation which exists between individuals in the study. It is noteworthy, we believe, that the first component, in view of the near uniformity of the elements of the eigen-vector, amounts to little more than the multivariate mean. The second component, on the other hand, as is apparent from the distribution of positive and negative signs, classifies the traits into (a) head and (b) not-head. The third component does not lend itself to simple interpretation, but we note that it corresponds roughly to a division along linear or longitudinal versus transverse or circumferential measures. These components are particularly interesting in view of an earlier study of body measurements of British adult males (Burt and Banks, 1947). These authors with a factor analysis, a procedure closely allied to principal components, isolate a "factor" for general body size, which accounts for over 50 per cent of the total variance. Their second "factor," contributing about 13 per cent to the total variance, classifies traits into longitudinal, on the one hand, and transverse, on the other. Our first and third components are, then, substantially the first and second reported by Burt and Banks. Our second component they do not report, but then none of the nine measurements used in their analysis involved the head. In view of the similarity between the findings on the British adult males and the young Japanese females, the components under discussion would appear to be relatively invariant from group to group.

In table 6 we give the regression coefficients relating the first three components to age and F' . With respect to age, we note that not only is the vector of regression coefficients significantly different from a zero vector ($\chi^2 = 1,172.20$; $df = 3$), but every element in the vector also differs from zero. However, the effect of age appears more pronounced on the first component, the "general body size factor." With respect to F' , neither the vector nor any of its elements differs significantly from zero ($\chi^2 = 5.02$; $df = 3$). It is intriguing, nevertheless, to note that all elements in the F' vector are negative, and that inbreeding would seem to have, relative to age, a greater effect on the second than the first component. Be this as it may, as judged by tests of significance the two analyses are in substantial agreement. It is also worth noting that if the change in mean phenotypic value per ten per cent increase in F' is expressed

TABLE 6. THE MEANS OF THE FIRST THREE LINEAR COMPONENTS PREDICTED FOR TEN-YEAR-OLD CHILDREN WHOSE COEFFICIENT OF INBREEDING FOR SEX-LINKED GENES IS ZERO. THE INCREMENTS OR DECREMENTS OF CHANGE IN THESE COMPONENTS PER MONTH OF AGE AND PER PER CENT F' ARE INDICATED AS ESTIMATED FROM THE POOLED CITY DATA

Variable	Mean	Regression Coefficients	
		Age	F'
First component	1502.13	4.0716*	-0.2143
Second component	228.45	1.9004*	-0.1867
Third component	-19.29	-0.1229+	-0.0123

*Significant at one per cent level.

+Significant at five per cent level.

as the percentage of the non-inbred mean, the two methods of analysis are, again, in agreement. What differences obtain, however, lead to larger estimates of the consanguinity effect through component analysis than through the observable traits.

It is important to bear in mind that two different, albeit related questions are at issue here. They are: (1) Is there evidence for the existence of one or more sex-linked "major" modifiers of the polygenic systems underlying the traits under consideration? (2) Does the X-chromosome participate in these polygenic systems in proportions to its length, or more properly, its total genetic information? We have seen that the data do not support the notion of "major" sex-linked modifiers. But what bearing do these data have on the second question? A clear and unequivocal relationship can not be established; however, some notion of their bearing emerges from the following consideration: Elsewhere (Schull, 1962) we have presented observations which suggest that the inbreeding depression, for these anthropometric measurements, varies from 1-5 per cent when the depression per ten per cent F is expressed as a percentage of the non-inbred mean. If we assume that the polygenes are distributed more or less at random over the chromosomes, we might expect some 6 per cent or so to be on the X-chromosome. This might, therefore, lead one to expect the inbreeding depression due to X-linked genes to be but a sixteenth or so of the depression due to autosomal loci. As is clear from tables 1 and 3, the data are consistent in general, or perhaps it should be said that they are not strikingly inconsistent, with this argument. Thus, we can not exclude the possibility that the X-chromosome participates in these polygenic systems.

SUMMARY

An effort to relate ten anthropometric measurements obtained on 853 daughters of first cousin spouses to the coefficient of inbreeding for sex-linked genes, F' , proved unsuccessful. Both the observable traits and the first three principal components fail to relate to F' . There is thus no evidence to support the existence of one or more "major" sex-linked modifiers of the systems of genes reflected in the traits under consideration. We can not, however, exclude the possibility that the X-chromosome participates in the polygenic systems responsible for the traits under measurement.

ACKNOWLEDGMENTS

This work is a portion of the Child Health Survey sponsored by the U. S. Atomic Energy Commission, the Rockefeller Foundation, the Association for Aid to Crippled Children, and the U. S. Public Health Service's National Institute of Dental Research. The Atomic Bomb Casualty Commission, a research agency of the National Academy of Sciences-National Research Council and the Japanese National Institute of Health, contributed data, facilities, and personnel in Hiroshima and Nagasaki. The unqualified endorsement of the Child Health Survey by the Subcommittee on Consanguinity Effects of the Japan Science Council's Committee on Genetics contributed materially to the success of this Survey. Analysis of the data was carried out under a grant of the Atomic Energy Commission to the University of Michigan, Contract AT(11-1)-942. We are indebted to Professor J. F. Crow for several thoughtful suggestions for the improvement of this paper.

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William Allan Memorial Award



NEWTON E. MORTON
WILLIAM ALLAN MEDALIST, 1962

The William Allan Memorial Award is designed to honor those who have made outstanding contributions to Human Genetics through research, or teaching, or both. Upon recommendation of the Awards Committee of the American Society of Human Genetics, the Award was presented, in absentia, to Dr. Newton E. Morton at the annual meeting of the Society held at Oregon State University, Corvallis, Oregon, on August 30, 1962. The Award recognizes Dr. Morton's outstanding contributions in statistical research, in teaching, and in service to the Society. Dr. Morton was until recently a member of the Department of Medical Genetics of the University of Wisconsin, but will assume new duties in 1963 at the University of Hawaii.

Book Reviews

INTRODUCTION TO IMMUNOCHEMICAL SPECIFICITY. By WILLIAM C. BOYD.
New York: Interscience Division of John Wiley and Sons, Inc., 1962.
(158 pp., \$8.00.)

Dr. Boyd's excellent book is a valuable contribution to the understanding of immunogenetics. He covers the subject clearly and succinctly and presents much more than an introduction to the area. Even those readers with considerable experience in immunochemistry will find the book worth reading. In some respects, this book is less detailed than other books on the subject; however, this is precisely what is needed in an area expanding as rapidly as is immunochemistry. The nature of antigens is thoroughly discussed as is the current status of knowledge about antibodies and lectins. In general, only an elementary understanding of biochemistry is expected of the reader; there are two chapters, however, dealing with antigen-antibody reactions, for which some background in physical chemistry would help, but is by no means essential. This book should be read by all geneticists who wish to expand their understanding of immunogenetics; teachers of intermediate and advanced genetics courses will find it a valuable supplement to a genetics text-book. Dr. Boyd should be congratulated for providing such a well-written volume for such a wide range of readers, but then again, perhaps no less should be expected of him.

EMANUEL HACKEL
*Department of Natural Science,
Michigan State University,
East Lansing, Michigan*

MUTATIONS, SECOND CONFERENCE ON GENETICS, JOSIAH MACY JR. FOUNDATION. Edited by William J. Schull. Ann Arbor: University of Michigan Press. 1962 (248 pp., \$6.00).

The Macy Conferences deal with three aspects of a central subject of current interest in biology and medicine. Each day a discussion leader attempts to present pertinent material and to call on other participants for supplementary discussion and comments. The presentations are subject to continuous interruptions in the form of questions and comments, and the report of each conference is essentially the stenographic transcript of everything said at the meeting. The conference topics for this meeting were:

First day: Problems of Measurement of Mutation Rates, presented by K. C. Atwood. This material covered the general question of the measurement of mutation rates with specific emphasis on man. The main experimental data presented were those from Atwood's studies on variations in human red blood cell types found in single subjects.

Second day: Chemical Mutagenesis, presented by C. Auerbach with supplementary presentations by S. Benzer on Genetic Fine Structure, M. Demerec on Mutagenic Effects of Manganese Chloride, and E. Freese on Introduction of Mutations with Base Analogues.

Third day: Mutagens of Potential Significance for Man, presented by A. Goldstein. This material dealt with the possible mutagenic nature of the many compounds to which man is now being exposed, the emphasis being placed on the possible importance of caffeine as a human mutagen.

As a participant at a recent Macy Conference on Genetics, I am very much aware of the value of these meetings for the participants. The spontaneous nature of the presentations and discussions, made possible by the small group attending, adds tremendously to the value of the meeting. This same spontaneity, however, becomes a liability when translated from the oral to the written form. The constant interruptions, interjections, and interpolations which can be both illuminating and even entertaining for the participants make it a chore for the reader to find his way through the resultant maze. On the other hand, there are many remarks and statements recorded which would not be found in usual publications and the persevering reader may well benefit from them. The subjects covered in this volume are of the greatest interest for human geneticists. While there is marked discontinuity in the coverage of the subjects, the quality of the presentations and discussions is of the highest order.

STANLEY M. GARTLER
*Department of Medicine,
University of Washington,
Seattle, Washington*

GENETICS. By E. GRACE WHITE. New York: Vantage Press, 1962. (258 pp., \$6.00)

GENETICS is a revised edition of a text published by C. V. Mosby Company in 1940 under the title *Principles of Genetics*. The author has added chapters on Neurospora and bacterial genetics and updated the information on the structure of the gene. The description of mitosis and meiosis is very abbreviated and many terms are introduced with meager explanation. The chapter on monohybrid and dihybrid crosses is very short with inadequate space given to the mechanics of the cross as compared with the amount of space allocated to variation in the ratios, *i.e.*, epistasis, lethals, etc. The ratios 3:1 and 1:2:1 are introduced early in this chapter before a monohybrid cross is discussed. This would seem to make it difficult for a beginning student to comprehend during an initial reading. In the chapter on quantitative inheritance and multiple alleles the explanation of the Rh factor uses Weiner's symbolism to demonstrate the theory of Fisher, Race and Sanger.

There is a dearth of mathematical material and no explanation of probability. Some statements do not seem to be consistent. In the chapter on multiple births, "It is characteristic of identicals that all die or all remain alive, since they all have the same amount of viability," and one paragraph later, "Even

in identical sets there is much difference in size and strength at birth" The chapter on human inheritance has not been revised to include recent advances, such as chromosomal abnormalities or biochemical work. Such statements as, "The loss of mentality results in insanity" are meaningless. The chapter on eugenics is short but important and if newer ideas were included would be more useful. The reviewer finds this book a second edition of the older book with very little material added since the late 1930's and with insufficient genetic information throughout.

JAMES V. HIGGINS
Department of Zoology,
Michigan State University,
East Lansing, Michigan

Epidemiology and genetics of mental deficiency in a southern Swedish population. By HANS O. ÅKESSON. Uppsala: Almqvist and Wiksells, 1961. (107 pp.)

This brief monograph from the Institute for Medical Genetics presents results of a census for mental deficiency in 10 randomly selected rural parishes with a population of 7,533 persons. Sex chromatin studies were performed on 88 and tests for phenylketonuria on 89 of 132 probands detected. As anticipated, no abnormalities were detected with either test. The frequency of mental retardation (Stanford-Binet form L < 68) was 1.8 ± 0.2 per cent. Morbid risks (uncorrected for differential mortality) were calculated for parents and sibs and were high relative to most previous studies. The geographical distribution of probands was heterogeneous and directly correlated with migration rates out of the parishes. (H. O. Goodman)

Cell Heredity. By RUTH SAGER AND FRANCIS J. RYAN. New York: John Wiley & Sons, 1961. (411 pp., \$7.50).

A textbook of genetics that limits itself to the molecular and cellular levels is a new departure, and emphasizes the growing importance of molecular biology. The presentation begins with the evidence to identify DNA as the basic hereditary material, discusses the early transformation experiments, and proceeds to a discussion of the principles of mutation and recombination at a chemical level. Chromosomal structure and cytogenetics are discussed at the level of structures visible with the microscope. While this is not to be considered a textbook of microbial genetics, the experimental material is largely drawn from this field. There are extensive sections on recombination in viruses and bacteria, and on functions of bacteriophage. Nonchromosomal inheritance is discussed in some detail. The authors have emphasized the areas of uncertainty that exist in molecular genetics by the inclusion of a number of concepts that are still in considerable doubt, with adequate identification for the student of topics that were uncertain or controversial as of 1960.

It is pointed out in the preface that population genetics and cell genetics have become so divergent in methodology that the authors consider separate presentations preferable. Population genetics is not considered in this volume. There is no attempt to relate the molecular and cellular principles discussed to practical applications of genetics to medical and agricultural problems. Also lacking is a systematic discussion of principles of evolution. These omissions are deliberate, and simply emphasize the specialized and restricted scope chosen by the authors. The book is well written and should prove useful as an introduction to molecular genetics for students and as a reference for geneticists whose primary interest is at other levels. (C.N.H.)

The Use of Vital and Health Statistics for Genetic and Radiation Studies: Proceedings of the Seminar Sponsored by the United Nations and the World Health Organization held in Geneva, 5-9 September, 1960. Edited by Staff, United Nations. New York: Office of Conference Services, United Nations, 1962. (259 pp., \$7.50).

Data derived from the vital statistics records of various countries have been used in genetic studies from time to time, but the limitations and uncertainties inherent in such data have been obvious. This Seminar brought together 63 participants representing various official agencies concerned with vital and health statistics, and a representative group of geneticists and epidemiologists. In the course of 9 sessions, 23 papers were presented dealing with the use of vital statistics and survey techniques for the study of genetic problems and particularly for the detection of radiation effects. Abstracts of the discussions of each session are presented, as well as summarizing closing remarks by the session chairmen. The General Chairman of the Seminar, Dr. Curt Stern, presented a closing address that summarized the problems touched upon and suggested possible solutions. The participants agreed upon a statement of consensus of opinion that includes 10 recommendations concerning improvement of vital statistics procedures that would make these more generally useful for genetic studies. A wealth of genetic and epidemiologic information is summarized in the formal papers. (C.N.H.)

Ideas on Human Evolution: Selected Essays, 1949-1961. Edited by WILLIAM HOWELLS. Cambridge: Harvard University Press, 1962. (555 pp., \$10.00).

The riddle of man's ancestry has stimulated much speculation and debate, and various authorities have made different interpretations of the relatively meager fossil material available. A number of changes in interpretation have occurred within the past 15 years, spurred both by new finds of fossil material and by development of new concepts. The author has collected 28 excellent papers that present many opinions concerning interpretation and concepts of evolution. Dr. Howells is a distinguished anthropologist, and it is to be expected that his choices would be skillful and would present various shades of opinion on controversial matters. The papers are all well written and the entire book makes enjoyable reading. (C.N.H.)

Ionizing Radiations and Immune Processes. Edited by CHARLES A. LEONE, New York: Gordon and Breach Science Publishers, 1962. (518 pp., \$12.50).

The eighteen papers presented in September, 1961, at an International Symposium jointly sponsored by the University of Kansas and the U. S. Atomic Energy Commission form the subject matter of this volume. It has been established that irradiation depresses the ability of the body to react to antigenic stimuli. Six papers are concerned with the effects of radiation on the physical, chemical, and biological properties of proteins, viruses, and cells. Four reports are concerned with modification of antibody production by radiation. It appears that irradiation of protein produces disruption of secondary structure, and this is apparently responsible for inactivation of certain enzymes. The depressing effect upon the ability of certain cells to form new enzyme systems seems greater than the effect on enzyme systems in operation at the time of irradiation. If antibody formation requires activation of new enzyme systems, at least one aspect of the mechanism of radiosensitivity of immune processes may have been identified. Four papers discuss the modification of irradiation effects by various drugs, and these seem to offer the prospect of specific chemotherapy for radiation sickness. Three additional papers are concerned with problems of bone marrow transplantation, and the final report is a review of 190 publications from the Russian literature concerned with these problems. The research described in all papers is adequately illustrated with charts and tables of data, full bibliographies follow each paper, and the entire volume is adequately indexed. (C.N.H.)

Recherches sur la structure et les fonctions des Acides Desoxyribonucleiques: Etudes Genetiques et Chimiques. By RENE THOMAS with preface by JEAN BRACHET. (No. 21, Actualites Biochimiques, edited by Marcel Florkin and Jean Roche) Paris: Masson et Cie., 1962. (104 pp., 24 NF)

This paperbound monograph is divided into three parts. The first considers the evidence concerning the structure and spatial configuration of the DNA molecule. The second part is concerned with the genetic function of DNA and summarizes the chemical aspects of experiments in bacterial transformation. The third part is concerned with evidence concerning reduplication of the DNA molecule. (C.N.H.)

Studies in Genetics. II. Research Reports on Drosophila Genetics, Taxonomy and Evolution. Edited by MARSHALL R. WHEELER. Austin: University of Texas. (554 pp.)

Reports on research in *Drosophila* genetics carried out at the Genetics Foundation of the University of Texas are presented in this paper bound volume. There are 18 research reports by 14 investigators. The topics covered include radiation effects, cytological and morphologic studies of various subgroups, and studies concerning phylogeny and natural selection. (C.N.H.)

Textbook of Virology for Students and Practitioners of Medicine. By A. J. RHODES AND C. E. VAN ROOYEN. Baltimore: Williams & Wilkins Company, 1962. (600 pp., \$13.50).

The Fourth Edition of this text has appeared only four years after the Third Edition, and has been largely rewritten. The orientation is primarily clinical, and 435 pages are occupied by short chapters, each dealing with a specific viral disease. The biologic properties of viruses, immunology, epidemiology, and technical methods are all covered in the first 134 pages. The section dealing with genetics and biochemistry of viruses seems inadequate. This revision should be useful to clinicians. (C.N.H.)

Design and Function at the Threshold of Life: The Viruses. By HEINZ FRAENKEL-CONRAT. New York: Academic Press, 1962. (117 pp., \$1.95, paperbound.)

The "educated layman" is the acknowledged target of this introduction to the biochemistry of viruses. The author is Professor of Virology at the University of California at Berkeley, and is well-known for his biochemical research. He has produced a well-written and interesting introduction to the chemistry of proteins and RNA with emphasis upon studies of the tobacco mosaic virus. The diagrams and illustrations used are simplified, but are adequately instructive. The book should be useful to those who desire an introduction to the subject. (C.N.H.)

Quantitative Chemical Techniques of Histo- and Cytochemistry. By DAVID GLICK. Volume 1. New York: Interscience Publishers, 1962. (470 pp., \$14.50)

This is the first half of a two-volume reference work in this rapidly expanding field. The author points out in the preface that a number of monographs now exist that are concerned with the techniques of qualitative histochemistry and with morphologic localization and identification of various compounds. Much less attention has been devoted to quantitative chemical techniques for the study of morphologically identified microsamples of cells or tissues. This volume provides a detailed presentation of the available quantitative techniques. The first three chapters are concerned with methods of preparation of biological samples, methods of measurement of samples, and descriptions of general apparatus and techniques of manipulation. The remaining four chapters consider in detail gasometric, electrometric, dilatometric and fluorometric techniques. The presentations are clear and detailed, and documented by a 24-page bibliography. Quantitative cytochemical techniques have been used to some extent in genetic studies, but it would seem that these techniques could be applied more extensively in human genetic research. (C.N.H.)

Darwin, Marx, and Wagner: A Symposium. Edited by HENRY L. PLAINE. Columbus: Ohio State University Press, 1962. (165 pp., \$3.50).

It is a historical coincidence that the chief works of Charles Darwin, Karl Marx, and Richard Wagner appeared in 1859. At first sight it may seem that *Origin of Species*, *Critique of Political Economy* and *Tristan and Isolde* have little in common, but in the succeeding century each had an important impact upon the cultural and intellectual scene. This volume presents seven essays evaluating these effects that constituted a centennial symposium held at Ohio State University. The historical evaluation of Darwin's impact upon modern biology by Bentley Glass will be of particular interest to geneticists, but the entire volume will also prove rewarding. This volume is recommended to those interested in the philosophy and historical impact of new ideas. (C.N.H.)

Psychology: A Study of a Science. Volume 4. Biologically Oriented Fields: Their Place in Psychology and in Biological Science. Edited by SIGMUND KOCH. New York: McGraw-Hill Book Company, 1962. (731 pp., \$12.50).

This is Volume 4 of a projected 7-volume series sponsored by the American Psychological Association. This volume develops the interrelationships between psychology and other areas of biological science. The first chapter is entitled "Some interrelations between psychology and genetics," contributed by Paul R. David and Laurence H. Snyder. Thirteen additional chapters by seventeen contributors discuss numerous interactions between psychology and various aspects of neurophysiology. The presentations are uniformly scholarly and well documented, with adequate references and with a detailed index to the entire volume. This should become an important reference work in this field. (C.N.H.)

Fundamentals of Psychology. By FRANK A. GELDARD. New York: John Wiley & Sons, 1962. (437 pp., \$7.50).

This is an introductory text book of general psychology for college-level courses. The text is well written and is profusely illustrated with photographs, diagrams and charts. A short list of recommended references for collateral reading follows each chapter. A search of the index for references to genetic components in behavioral problems reveals only a brief discussion of "heredity vs. environment," in which heredity promptly comes off second best. (C.N.H.)

Handbook of Physiology. Section 2: Circulation, Volume 1. Edited by W. F. HAMILTON AND PHILIP DOW. Washington: American Physiological Society, 1962 (Distributed by Williams and Wilkins, Baltimore). (758 pp., \$24.00).

This is the first of three projected volumes of the section dealing with the circulation in the multivolume handbook of physiology. The physiology and biophysics of the blood and its exchangeable fluids, and the action and control of the heart are covered in this volume. As would be expected, each of the 22 chapters written by 30 contributors discusses a particular aspect of circulatory physiology in great depth. The entire series is designed as a reference work, and each volume contains a detailed reference list and detailed index. This volume does not include genetic discussions as such, but the material is significant to geneticists. The basic information provided concerning normal physiology and biophysics is obviously essential to studies of genetic factors influencing the cardiovascular system. This series should become a standard reference work that will be useful for many years. (C.N.H.)

Actions of Radiations on Living Cells. By D. E. LEA. New York: Cambridge University Press, 1962, Reprint of Second Edition. (416 pp., \$2.95, paper bound).

The first edition of this monograph, published in 1947, was a comprehensive and judicious summary of the information then available concerning the effects of ionizing radiations on living systems. Unfortunately, the second edition in 1955 was not rewritten, and the two editions are identical for the first 363 pages. An appendix of 11 pages, plus 2 pages of references, was added to report the developments of nearly a decade, and was inadequate. The paperback version is a reprint of the second edition. (C.N.H.)

ANNUAL MEETING
 AMERICAN SOCIETY OF HUMAN GENETICS
 July 19, 20, 21, 1963
 New York City, Hotel Americana in conjunction with
 Second International Conference on Congenital Malformations,
 July 15-19, 1963

STATEMENT of the ownership, management, and circulation required by the Act of Congress of August 24, 1912, as amended by the Acts of March 3, 1933 and July 2, 1946 (Title 39, United States Code, Section 233), of THE AMERICAN JOURNAL OF HUMAN GENETICS published quarterly at Jacksonville, Fla. for January 1, 1963. 1. The names and addresses of the publisher, editor, managing editor, and business manager are: Publisher, Grune & Stratton, Inc., 381 Park Avenue South, New York 16, New York; Editor, C. Nash Herndon, M.D., Bowman Gray School of Medicine, Winston-Salem, North Carolina; Managing Editor, Duncan Mackintosh, 381 Park Avenue South, New York 16, New York; Business Manager, Arthur Murat, 381 Park Avenue South, New York 16, New York. 2. The owner is: (if owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership, or other unincorporated firm, its name and address, as well as that of each individual member, must be given.) American Society of Human Genetics, (office of the Secretary, Dr. Samuel H. Boyer) Johns Hopkins Hospital, Baltimore, Maryland. 3. The known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, or other securities are: (If there are none, so state.) None. 4. Paragraphs 2 and 3 include, in cases where the stockholder or security holder appears upon the books of the company as trustee or in any other fiduciary relation, the name of the person or corporation for whom such trustee is acting; also the statements in the two paragraphs show the affiant's full knowledge and belief as to the circumstances and conditions under which stockholders and security holders who do not appear upon the books of the company as trustees, hold stock and securities in a capacity other than that of a bona fide owner. 5. The average number of copies of each issue of this publication sold or distributed, through the mails or otherwise, to paid subscribers during the 12 months preceding the date shown above was: 1580. Signed: Duncan Mackintosh, Managing Editor. Sworn to and subscribed before me this 1st day of January, 1963. (Seal) Louise E. Hoffheimer (Moskow). (My commission expires March 30, 1963.)

Mutation Rates of the Abnormal Hemoglobin Genes

O. FROTA-PESSOA AND ANITA WAJNTAL

*Department of Biology,
University of São Paulo,
São Paulo, Brazil*

MODERN EVIDENCE shows that the sickle-cell gene is maintained in African populations in a state of balanced polymorphism as a result of the heterozygotes being at an advantage in malarious areas (Allison, 1954a). Since this advantage can not be determined with great accuracy, it is impossible to evaluate the mutation rate for this gene from African data; practically any mutation rate is compatible with them. A population where heterozygotes and normal homozygotes have equal adaptive value is ideal for determining the mutation rate. Such a population is not known for sure at present, but data are available for populations approaching this condition. The advantage of the heterozygotes tend to increase their frequency, therefore a population having the smallest known frequency of heterozygotes is probably the best approximation to consider.

Large samples studied for the detection of heterozygotes outside of Africa are rare. Eng (1953) examined "more than 4,000 samples of blood from different islands in Indonesia" by paper electrophoresis and found two heterozygotes for the sickle-cell gene (hemoglobin S). So far as known, one of them was of pure Indonesian descent and the other had only Chinese-Indonesian ancestry. These are the first cases reported from Indonesia.

Under the limitation of a few assumptions, these data allow an evaluation of the mutation rate for the sickle-cell gene. Taking the frequency of heterozygotes in Indonesia as being $2q(1 - q) = 5 \times 10^{-4}$ and assuming equilibrium, the frequency of the sickle-cell gene is $q = 2.5 \times 10^{-4}$ and the frequency of abnormal homozygotes is $q^2 = 6.25 \times 10^{-8}$. Adopting a selection coefficient against the abnormal homozygote equal to $s = 0.75$, as estimated by Allison (1954b), the mutation rate (equal to the elimination) will be $q^2s = 5 \times 10^{-8}$.

The present estimate is based on certain assumptions which must be discussed. If, as suggested by Eng (1953), the abnormal gene were derived from crosses with populations (such as African) where its frequency was influenced by balanced polymorphism our result would be an overestimate. On the other hand, should the adaptive value of heterozygotes in absence of balanced polymorphism be inferior to normal, our evaluation would be an underestimate. This is a critical point since a small inferiority of the heterozygotes (of about 0.02) would be enough for shifting the calculated mutation rate to about 10^{-5} . Evidence on the adaptive value of heterozygotes for the sickle-cell gene outside malarious zones is inconsistent (Rucknagel and Neel,

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1961). However, we may equally suspect that heterozygotes have an adaptive value lower than normal homozygotes in the case of most recessive traits for which mutation rates have been calculated. By assuming, as it is usually done for all cases, equality in adaptive value between heterozygotes and normal homozygotes, we risk underestimating the mutation rate, but we can compare its order of magnitude to the rates available for other genes. We also assumed that the penetrance of the sickle-cell gene is complete or nearly so and indeed, as shown by Rucknagel and Neel (1961), the available evidence supports this assumption. It seems, therefore, that our evaluation although inaccurate probably is not much more so than current determinations by the indirect method (see Penrose, 1956).

It is an interesting fact that Silvestroni *et al.* (1958) in a sample of 2,186 individuals from Southern Italy, including Sicily and Sardinia, found one heterozygote for the gene of each of the following abnormal hemoglobins: D, J, K and G (or Q). This is practically the same frequency as found by Eng for the sickle-cell heterozygotes and it may be concluded that the mutation rates for all these abnormal hemoglobin genes are very low. Heterozygotes for the gene of hemoglobin S in Silvestroni's sample had a frequency about four times greater (4 in 2,186) than that found by Eng. This, however, does not necessarily mean that the mutation rate for this gene is higher in Italy than in Indonesia, since the higher frequency of heterozygotes may be due to the prevalence in the past of endemic malaria in certain areas of Italy. Moreover, the frequencies in Eng's and Silvestroni's sample do not differ significantly ($\chi^2 = 2.6$; $P > 0.10$). The mutation rate calculated from the pooled data becomes 1.8×10^{-7} .

Mutation rates have been evaluated for about 30 different genes in man. All values are greater than 10^{-6} with three exceptions: facioscapulohumeral muscular dystrophy (5×10^{-7} , Morton and Chung, 1959), Huntington's chorea (Reed and Neel, 1959) for which an upper limit was set at 10^{-6} , and "Porcupine" (Penrose, quoted in United Nations, 1958) for which an upper limit was set at 10^{-9} as a very rough estimate. Penrose (1956) suggested that a rate of about 5×10^{-6} may be taken as representative for dominants. Estimates for sex-linked and for recessive genes are always greater than that (Penrose, 1956). In sharp contrast with this, these calculations lead to an estimate of 5×10^{-8} for mutation rates to the gene of hemoglobin S and suggest that mutation rates to other abnormal hemoglobin genes are similarly low. This raises the suspicion that estimates of mutation rates cluster around two orders of magnitude, one "high" (10^{-5}) and the other low (10^{-8}). If this is true, the reason for it could be related to the distinction (Muller, 1950) between mutations to amorph and to neomorph alleles. The first type (*e.g.*, mutation to hemophilia A and B, albinism, phenylketonuria) alters the gene in such ways that no specific gene product is detectable; hence an amorph allele is detected only through the absence of the gene product (or phenotype) typical of the normal allele. The second type of mutation (*e.g.*, mutation to hemoglobin S) gives rise to an allele which promotes a specific recognizable product, qualitatively different from the product of the normal allele.

It is possible that most amorphs are not truly inactive. Since their products are unknown, different alterations in the gene molecule, leading to different undetectable products, can not be distinguished from each other. All of them, together with small deletions involving the locus, are taken as being the same mutation. This tends to raise the estimates of mutation rates for amorphs in relation to the estimates for neomorphs, where a specific alteration of the gene molecule is distinguished from others because it promotes a recognizable product.

Based on similar considerations, Rucknagel and Neel (1961) pointed out that "on theoretical grounds alone, we may expect the rate of mutation resulting in any specific abnormality of hemoglobin to be well below the usual estimates," and suggested as readily conceivable that the mutation rate is of the order of 10^{-8} for a specific hemoglobin type. The value here calculated from Eng's data confirms this expectation.

SUMMARY

From populational data by Eng (1953) a value of 5×10^{-8} has been estimated for the mutation rate to the sickle-cell gene. The sources of error of this estimate are discussed. The possibility that mutation rates cluster around two orders of magnitude (10^{-5} and 10^{-8}) has been considered and this has been related to the occurrence of two kinds of mutations: those to amorph alleles and those to neomorph alleles.

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Glucose-6-Phosphate Dehydrogenase Deficiency in Taiwan

TING-CHIEN LEE,¹ LING-YU SHIH,¹ PAI-CHI HUANG,¹
CHI-CHUH LIN,¹ BOON-NAM BLACKWELL,²
R. QUENTIN BLACKWELL² AND DAVID YI-YUNG HSIA³

¹*Department of Pediatrics, College of Medicine,
National Taiwan University,
Taipeh, Taiwan*

²*Namru-2, Taipeh, Taiwan*

³*Genetics Clinic, Children's Memorial Hospital
and Department of Pediatrics,
Northwestern University Medical School,
Chicago, Illinois*

GLUCOSE-6-phosphate dehydrogenase (G-6-P.D.) deficiency is recognized to exist with relatively high frequency among several ethnic groups, notably Negroes and Caucasians of Mediterranean origin including Sardinians and Sephardic Jews (Motulsky, 1960). Relatively few reports have been made concerning the G-6-P.D. deficiency rate among Mongolian peoples (Beutler, Yeh and Necheles, 1959; Vella, 1959; Motulsky, 1960; Smith and Vella, 1960; Weatherall, 1960; Kruatrachue and Harinasuta, 1961). The present paper describes the presence of this enzyme defect among the Chinese residents of Taiwan; the first section deals with the incidence of reactors in the general population and the second with a genetic study of eight affected families. Preliminary reports concerning portions of the present work have appeared in the Chinese literature (Blackwell *et al.*, 1961; Shih and Lee, 1961).

MATERIALS AND METHODS

The current Chinese population of Taiwan may be divided conveniently into three groups on the basis of their earlier mainland China origin and time of migration to Taiwan: Taiwanese Chinese, Hakka Chinese, and Mainland Chinese. Taiwanese Chinese, currently numbering approximately nine million, are descendants of emigrants who left the mainland principally during the seventeenth, and to a lesser extent during the eighteenth and nineteenth centuries. The vast majority of those emigrants were from Minnan County of Fuchien Province which is situated along the southeast coast of mainland China.

The Hakka Chinese people are a much smaller group of several hundred thousand whose ancestors came to Taiwan primarily during the sixteenth and seventeenth centuries. They emigrated from Kwangtung Province along the southern coast of China. The Hakkas were a separate group among the residents of Kwangtung Province and lived primarily in three counties: Chiayin Chou,

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Huei Chou, and Chhau Chou. The present Hakka population of Taiwan springs from emigrants from all three counties. Up to the present time the Hakka people in Taiwan have continued to retain their own dialect and customs as well as to live generally in separate communities. Although the Taiwanese Chinese and Hakka Chinese have lived in Taiwan for more than three centuries, there has been little intermarriage so that the present population retains both distinct groups.

The Mainland Chinese population of Taiwan is composed of people who originally resided in many provinces of the Chinese mainland and who migrated to Taiwan primarily during the period 1948-1950, and in smaller numbers in the following years. This group, combined with their children born since their residence in Taiwan, totals approximately two million.

The remaining important group of Taiwan inhabitants are the aborigine people whose ancestors are believed to have arrived in Taiwan from Malaya and other parts of Southeast Asia more than a thousand years ago. Studies on this population, which is composed of at least eight distinct tribes, currently are in progress.

In the present study attention was directed toward the Chinese inhabitants of Taiwan and particularly toward the Hakka group because an increased incidence of reactors was found among them in a previous preliminary survey (Blackwell, 1961). In a recent study (Shih and Lee, 1961) of families affected by acute hemolytic anemia and showing G-6-P.D. deficiencies, several were from Hakka communities. The eight families used in the present genetic study were chosen from those located in the previous study, but none of the eight families are Hakka, all being either Mainland or Taiwanese Chinese families.

In the population study 3,236 individuals from the three Chinese groups were screened by means of the Motulsky dye test (Motulsky, Kraut, Thieme and Musto, 1959), all specimens failing to discolor after incubation for 55 minutes being considered enzyme deficient. In many cases, the results were confirmed by quantitative measurement of G-6-P.D. levels (Zinkham, Lenard and Childs, 1958).

For the family studies, blood samples were drawn from 46 members of eight affected families and from 62 controls (43 Taiwanese and 19 Mainland Chinese). Determinations were made for glutathione (GSH) concentration as well as glutathione stability in the presence of acetylphenylhydrazine (APH), according to the method of Stevenson, McDonald and Ruston (1960). G-6-P.D. quantitative analyses were made as described by Zinkham *et al.* (1958). The reactor status of each individual was determined by the combined results of these tests. The mean G-6-P.D. level of the controls was 194.1 ± 34.3 units/100 ml. RBC with a range of 140-322 units/100 ml. RBC. No significant difference was found between the mean levels of the Taiwanese (194.9 ± 32.8 units/100 ml. RBC) and the Mainlander (192.0 ± 37.6 units/100 ml. RBC) controls.

All family members whose G-6-P.D. level was below 3 standard deviations from the mean (90 units/100 ml. RBC or less) and whose GSH declined by more than 50 per cent after incubation with APH were classified as reactors. Those whose enzyme level was between 90-140 units/100 ml. RBC and

TABLE 1. FREQUENCY OF REACTORS AMONG VARIOUS POPULATION GROUPS IN TAIWAN

	MALES			FEMALES			ALL		
	No. Tested	No. Abn.	%	No. Tested	No. Abn.	%	No. Tested	No. Abn.	%
I. Mainland Chinese	282	5	1.77	163	2	1.23	445	7	1.57
II. Taiwanese Chinese	343	1	0.29	258	1	0.39	601	2	0.33
III. Hakka Chinese	1535	84	5.47	655	15	2.29	2190	99	4.52
a. Hsin-Pu (Hsin-Chu Hsien)	442	30	6.79	218	3	1.38	660	33	5.00
b. Hu-Kou (Hsin-Chu-Hsien)	242	16	6.61	78	4	5.13	320	20	6.25
c. Mei-Nung (Kaohsiung Hsien)	851	38	4.47	359	8	2.23	1210	46	3.80

whose GSH decreased by more than 50 per cent after incubation with APH were considered intermediate reactors and individuals with G-6-P.D. levels over 140 units/100 ml. RBC and less than 50 per cent GSH decrease after incubation with APH were classified as normal.

RESULTS

Population Studies

Table 1 shows the frequency of G-6-P.D. reactors among the three Chinese populations. It is apparent that there is a relatively low incidence of reactors among the Mainland and Taiwanese Chinese. However, among the Hakka Chinese the incidence of reactors was elevated with levels ranging from 4.5 to near 7 per cent among the males. In comparison, the number of enzyme deficient females detected in this population (2.29 per cent) is lower than might be expected.

It is particularly interesting that there is an increased incidence of glucose-6-phosphate dehydrogenase deficiency among one population group that migrated to Taiwan about 300 years ago, and no such increase in the other. Because it is not possible at the present time to undertake population studies on the Chinese mainland, one can only speculate regarding the cause for this difference. Two hypotheses present themselves. The first is that the Hakka people had an increased incidence prior to the time when they migrated to Taiwan and, because they remained as an isolated population group in Taiwan, their increased incidence has not changed appreciably over the centuries. In the same way, the Taiwanese Chinese came from another part of China where the incidence of reactors presumably was low and it has remained low since migration. The second hypothesis is that there have been selection pressures upon the Hakka people after their migration, whereas no such pressures have existed for the other migrants. This theory is less acceptable for two reasons. First, the period since migration has been relatively short for a significant change in frequency. Second, both the Hakka district in Kwangtung and the three counties where the Hakkas have lived in Taiwan are in low-lying areas, and there is good reason to believe that malaria existed in both areas until very recently. Therefore, it is unlikely that this was a factor in population selection. (Siniscalco, Bernini, Latte and Motulsky, 1961).

Family Studies

The pedigrees of the families investigated are illustrated in Fig. 1 and their erythrocyte G-6-P.D. levels and GSH stability tests in Fig. 2 and 3. Because seven of the 15 parents studied as well as 17 of 31 sibs are affected, a dominant mode of transmission is suggested. Among the parents six mothers and one father are affected, and the most common mating is that permitting the transfer of the gene to the offspring through the mother. This is the pattern expected with a common sex-linked, rather than an autosomal, gene and is demonstrable in families 1 to 6. In family 7, however, the father and one daughter are reactors, whereas the mother and other children are normal, although the female sibling must be a heterozygote. Again, in family 8, two sons are reactors,

the mother is normal, and the father is unavailable for testing. If the gene is sex-linked, the mother in both these families and the female sibling in family 7 must be intermediate reactors; since normal G-6-P.D. activity and glutathione

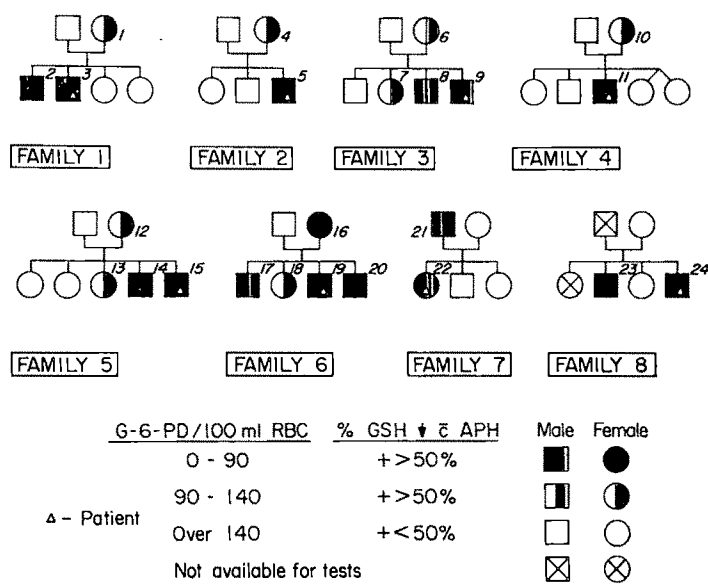


FIG. 1. Pedigrees of eight families of acute hemolytic anemia.

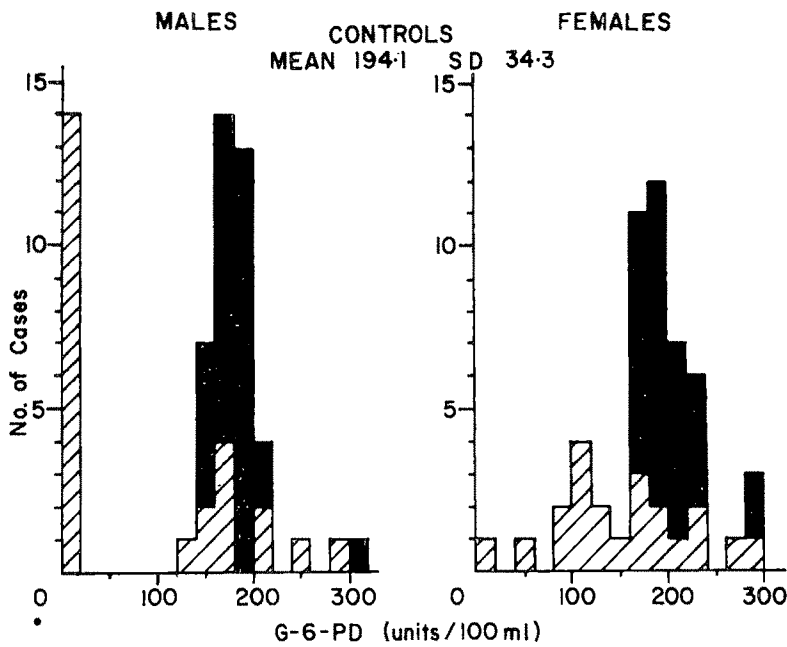


FIG. 2. Activity of glucose-6-phosphate dehydrogenase expressed as units per 100 ml. of packed erythrocytes. Cross hatched bars represent the patients and family members shown in Fig. 1 (eight families) and solid bars represent controls.

stability tests were obtained, variable penetrance in the female must be assumed. These data fit well with the hypothesis that G-6-P.D. deficiency is transmitted by a sex-linked gene showing incomplete dominance and variable expression (Gross, Hurwitz and Marks, 1958).

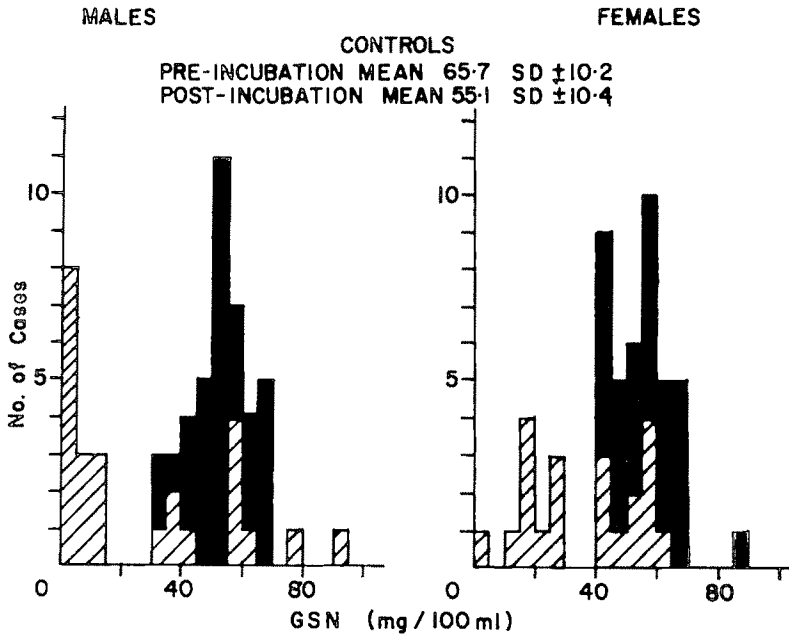


FIG. 3. Glutathione stability test of erythrocytes. Cross hatched bars represent the patients and family members shown in Fig. 1 (eight families) and solid bars represent controls, 31 males and 31 females.

SUMMARY

The present paper describes the presence of glucose-6-phosphate dehydrogenase deficiency among the Chinese residents of Taiwan. A population survey shows an increased incidence of reactors to between 6 and 7 per cent among males of the Hakka Chinese in Taiwan. Family studies have shown that the condition is transmitted by a sex-linked gene showing incomplete dominance and variable expression.

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Progressive Muscular Dystrophy. V. The Identification of the Carrier State in the Duchenne Type by Serum Creatine Kinase Determination

R. RICHTERICH, S. ROSIN, U. AEBI AND E. ROSSI

WITH THE TECHNICAL ASSISTANCE OF L. BRAUN

Institute for Medical Chemistry, the Children's Hospital, and the Department of Genetics of the Institute of Zoology of the University of Berne, Switzerland.

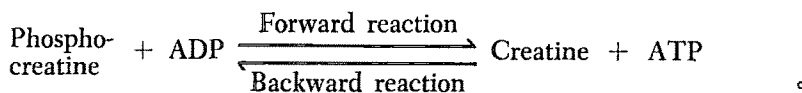
IN THE DUCHENNE form (type III) of progressive muscular dystrophy the activity of several serum enzymes (aldolase, lactic dehydrogenase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, phosphohexoseisomerase, creatine kinase) is elevated markedly. Comparative studies in affected children have shown that serum creatine kinase is superior to the other enzymes in discrimination between normal and type III dystrophy and between type III dystrophy and other forms of hereditary muscular dystrophy (Aebi, Richterich, Stillhart, Colombo and Rossi, 1961; Dreyfus and Schapira, 1962). Dreyfus and associates (Dreyfus, Schapira and Demos, 1960; Schapira, Dreyfus, Schapira and Demos, 1960; Dreyfus and Schapira, 1961; 1962) reported that the activity of this serum enzyme is elevated in some of the mothers of children with the Duchenne type dystrophy. Contrary to these observations, a Japanese group (Okinaka *et al.*, 1961) found normal serum creatine kinase values in the parents of five children with muscular dystrophy but elevated values in some female sibs of the affected boys.

Since creatine kinase activity is extremely low in normal sera these differences are possibly due to technical factors. Using a different method for serum creatine kinase assay we have investigated the enzyme levels in 438 members of 23 families of children affected with muscular dystrophy. A preliminary note on these studies has been published elsewhere (Aebi, Richterich, Colombo and Rossi, 1961/1962; Richterich, Aebi and Rossi, 1962).

METHODS AND MATERIALS

Assay of Serum Creatine Kinase

Creatine kinase (creatine phosphokinase) is the trivial name for ATP: creatine phosphotransferase (Report of the Commission, 1961) which catalyzes the reaction:



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TABLE 1. SUMMARY OF PATIENTS WITH MUSCULAR DYSTROPHY

Case No. ¹ and Sex	Age at examina- tion	Locali- zation ²	Pseudo- hyper- trophy	Function ³	Contrac- tures ⁴	SGOT ⁵ IU	Creatine kinase IU
PMD — Type I							
12 (m)	9	GOU	—	2	E	29	7.4
12a (m)	7	GOUS	—	7	SEIKF	7	1.1
PMD — Type II							
4 (m)	10	OU	—	1	—	14	0.7
	11			1	—	16	
7 (f)	9	SOU	—	3	—	23	0.3
	10			2	—	18	
10 (f)	7			4	EIF	15	5.9
	8	SOU	—	4	EIF	21	
	9			4	EIF	9	12.3
11 (f)	4			2	—	23	2.1
	5	SOU	—	3	—	96	
13 (f)	7			3	IF	19	3.6
	8	SOU	—	3	IF	22	
PMD — Type III							
1 (m)	8	OUS	(+)	6	EIKF	28	14.6
	11			10	SEIKF		
2 (m)	4	UOS	(+)	2		10	17.7
	19			11	SEIKF		
3 (m)	11	UOS	(+)	9	SEIKF	36	15.4
	12			12	SEIKF	36	
5 (m)	7	UOS	+	3	I	87	65.7
	8			4-5	EIF	59	
6 (m)	9	SOU	+	6	IKF	44	25.0
9 (m)	7			3	IF	86	
	8	SOU	+	7	EIKF	36	14.1
	9			7	EIKF		
14 (m)	7	OU	+	4	IF	79	66.0
	8			7	EIKF	81	
15 (m)	9	OU	+	4	I	83	24.5
	10			5	IKF		
16 (m)	7	SOU	+	4	IF	48	12.1
	8			5	SEIF		24.2
16E (f)	41	U	—	1			12.2
16F (m)	4	U	—	1			7.0
16G (m)	3	U	+	1		117	149.0
	4			1	I	43	69.5
16H (m)	1	—	—	0	—	76	160.0
	2			0	—	134	135.5
17 (m)	7	SOU	+	2	—	86	154.7
	8			4	IF	97	
18 (m)	16	SOU	+	8	SEIKF	33	
	17			9	SEIKF		
19 (m)	12	SOU	+	9	SEIKF	37	24.0
	13			10	SEIKF	14	
22 (m)	9	OU	+	6	EIKF	40	49.2
	10			6	EIKF		
26 (m)	7	SOU	+	3	IF	77	71.0
	8			4	IF	88	156.8
28 (m)	6	UOS	+	3	I		171.0
30 (m)	12	SOU	+	5	IF	87	135.8
31 (m)	10	SOU	+	3	IF	188	237.6
Normal range						4-18	0-1.8

¹Refers also to family and pedigree No.²Localization: G(face), O(arms), U(legs), S(trunk).³Thompson and Vignos (1959).⁴Contractures: S(shoulder), E(elbow), I(iliotibial tract), K(knee), F(feet).⁵Serum glutamic oxalacetic transaminase.

This enzyme is present in nervous and muscular tissues only (Colombo, Richterich and Rossi, 1962). Its activity in normal serum is about one million times lower than in skeletal muscle. A sensitive method must therefore be used for its determination.

The forward reaction occurs about 10 times faster than the backward reaction. Theoretically it is therefore more suitable for serum enzyme assays. Schapira and associates (Dreyfus *et al.*, 1960; Schapira *et al.*, 1960) incubated phosphocreatine and ATP, and measured the amount of creatine liberated. Attempts to use this method in our laboratory have not been successful and the experiences of Rotthauwe, Zurukzoghlu-Slavounou and Hammann (1961) have been similar. Another method which assays the forward reaction has been described by Oliver (1955). The ATP formed is measured in the optical test using the hexokinase and glucose-6-phosphate dehydrogenase system. In our experience this method is not suitable for assays of serum creatine kinase since serum contains considerable amounts of adenosine-triphosphatase (Meister, 1947). Wachstein and Sigismondi (1958) found approximately 15 International Units (IU) of adenosinetriphosphatase in 1,000 ml. serum at pH 9.3 and 37°C. The activity of this enzyme is therefore about twice as high as that of creatine kinase.

The Japanese workers (Ebashi, Toyokura, Momoi and Sugita, 1959; Okinaka *et al.*, 1961) used the backward reaction and measured the amount of phosphocreatine formed by determining the increase in "inorganic phosphate" during the incubation. This method is not specific and not sensitive enough for the exact determination of creatine phosphokinase in normal human sera. We have therefore modified the technique of Tanzer and Gilvarg (1959) for serum assays. The ADP formed is determined by the optical test using the pyruvate kinase and the lactic dehydrogenase system. The modifications include appropriate blanks (correcting for serum alkaline phosphatase and adenosinetriphosphatase activity), the use of more serum, and an increase of the incubation temperature from 25° to 37°C. The method has been described in detail elsewhere (Colombo *et al.*, 1962). During the last two years more than 2,000 assays have been performed and no technical difficulties were encountered. All results are expressed in International Units (IU), i.e., micromoles of substrate used per minute per 1,000 ml. serum at 37°C. (Report of the Commission, 1961).

Patients

All patients with progressive muscular dystrophy in care of the Children's Hospital were included in this study. No attempts were made to collect all cases in a particular area. Clinical data on most of these patients have been published elsewhere (Aebi *et al.*, 1961) but a summary is presented in table 1. If serum creatine kinase was assayed several times during one year the highest observed value is reported. The clinical stages are graded according to Thompson and Vignos (1959). No difficulties were encountered in the classification of patients according to the system of Walton and Natrass (Walton and Natrass, 1954; Walton, 1955; 1956) using the criteria of Chung and Morton (1959). Three exceptional cases deserve a more detailed description.

Case 16 E: Female, born 1920. Maternal aunt of cases 16, 16 H and 16 G. Had what was later said to be poliomyelitis with severe pneumonia at age five. No complete paralysis but slowly progressive weakness in both legs for about three to five years. Since then, stiff gait and difficulty in walking downhill. Leads the life of a mountain farmer's wife in very rough area about 1,000 m above sea level. Mother of four healthy children, of which the youngest, age five (*Case 16 F*), has some minor weakness of legs. Serum creatine kinase 12.0 IU. This case has been reported previously by Sidler (1944) as Case 4, and by Aebi *et al.* (1961/1962) as Case 4.

Case 16 H: Male, born March 25, 1960. Does not walk yet and does not sit up from prone position. Does sit by himself but rarely tries to pull himself up. Able to walk when holding to things at the age of 15 months. At that time no clinical evidence of muscular dystrophy. Serum creatine kinase 128.8 IU. This child has previously been reported as Case 23 by Aebi *et al.* (1961), and as Case 6 by Aebi *et al.* (1961/1962).

Case 16 F: Male, born 1955, son of Case 16 E. Suffers from weak legs. Not yet clinically investigated. Serum creatine kinase 7.0 IU. He has previously been mentioned as Case 7 by Aebi *et al.* (1961/1962).

Case 16 E will be considered as a biochemical carrier of type III dystrophy (clinical carrier), and her clinical findings will be discussed later. Case 16 H will be taken as suffering from type III dystrophy but still being in the preclinical stage. Case 16 F will be deleted since it is not certain if he is really suffering from muscular disease.

Field Study

Venous blood of as many relatives of these patients as possible was obtained either at the hospital or (mostly) at their homes. The blood was allowed to clot, and the serum was separated by centrifugation. The tube was labeled by a code known to the field investigator only, and frozen immediately in a box containing a salt-ice mixture. It was kept frozen until analysis was performed in the laboratory. The interval between sampling and analysis varied, from two weeks to two months in the earlier part, and from one to three days in the later part of the study. Since freezing causes a slight loss of activity (in the order of 0.2 — 0.5 IU, Colombo *et al.*, 1962) all samples were frozen to obtain uniform results. After all analyses were complete, the data were identified by the field investigator. The study was performed between September 1960 and May 1961.

RESULTS

Children with Muscular Dystrophy

The results of serum creatine kinase assays in 23 children with muscular dystrophy are presented in table 1. In two children with type I dystrophy enzyme levels were normal or slightly elevated. In the limb girdle type values may be normal or as high as 10 IU. All patients with the Duchenne type had creatine kinase values above 10 IU, ranging between 15 and 350 IU. One child who died had 16 IU one day prior to death (*Case 3*), thereby confirming our impression that even in advanced cases serum creatine kinase activity does not drop below 10 IU. The relationships among age, stage and enzyme values have been discussed elsewhere (Aebi *et al.*, 1961).

It should be mentioned that in polymyositis extremely high values (up to 1,000 IU) may be encountered. In our limited experience it has been observed that serum creatine kinase activity stays remarkably constant in patients with

dystrophy but fluctuates from week to week in polymyositis (Aebi, Friolet and Richterich, in preparation).

The per cent cumulative frequency of serum creatine kinase values of all children with type III dystrophy is presented in Fig. 7.

Range and Distribution of Normal Values

One family (No. 12) of two children with the facioscapulohumeral type (type I), five families (No. 4, 7, 10, 11, 13) with children of the limb girdle type dystrophy (type II), and 16 families (No. 1, 2, 3, 5, 6, 9, 14, 15, 16, 17, 18, 19, 26, 28, 30, 31) with at least one child affected by the Duchenne type dystrophy (type III) were investigated. All persons available, a total of 438 persons, were examined. The following three groups were removed from further consideration.

Pregnant females: Serum creatine kinase may rise to 3.2 IU toward the end of pregnancy (Colombo *et al.*, 1962). Results of analyses during pregnancy were therefore excluded. Two possible carriers had unusually high values during pregnancy (2.6, 6.6 and 5.8 IU; 19.0 IU). It is possible that the stimulus to the slight rise in normal pregnancy is more effective in carriers but more data will be necessary to substantiate this impression.

Newborns: During the newborn period serum creatine kinase activity may be as high as 12 IU (Colombo *et al.*, 1962). Results obtained from newborns were therefore deleted. It has been suspected that enzyme levels in newborns with muscular dystrophy may be exceedingly high (Aebi *et al.*, 1961) despite lack of clinical signs. Four newborns of possible carriers, but with creatine kinase activities below 1.8 IU, have been studied so far. All had normal serum enzyme levels and have since stayed within the normal range.

Children under six years of age: In early childhood (up to about six years) abnormally high values are encountered occasionally in apparently healthy children. Since serum creatine kinase has not yet been investigated systematically in young children such data have been deleted.

Dreyfus *et al.* (1960) did not observe an effect of physical exercise on serum creatine kinase levels. Contrary to their experience we found a marked influence of exercise or work on the activity of this serum enzyme. Our interest in the effect of exercise on serum enzyme activities dates back to a study on glutamic oxalacetic and glutamic pyruvic transaminase in soldiers (Richterich, Verrey, Gautier, and Stampfli, 1961). It was noted that glutamic oxalacetic transaminase values were on an average much higher in physically active troops (infantry) than in technical units. Since glutamic pyruvic transaminase values were similar in both groups it was assumed that the rise in glutamic oxalacetic transaminase was due to physical exercise or minor muscular lesions. More recently, we have observed that athletic training causes a rise in serum glutamic oxalacetic transaminase, aldolase, lactic dehydrogenase and creatine kinase (Baumann, Richterich, Escher and Schönholzer, 1962). After moderately severe training during three hours creatine kinase rose from about 1 to 4-6 IU. Elevated serum enzyme values normalized within 24 to 48 hours after exercise.

Since possible effects of physical exercise on serum creatine kinase values were anticipated, care was taken to obtain information on physical activity during the last 48 hours prior to blood sampling. For the establishment of normal values two groups were distinguished, one with a history of exercise (exercise positive) and one without hard physical work during the two days

prior to blood sampling (exercise negative). As expected, a positive history of exercise was obtained more frequently from males than from females (males: 41 positive to 148 negative; females: 10 positive to 106 negative). Elevations were usually mild, but after severe physical work (farmers or factory workers) values as high as 6 IU were encountered. Normal housework, office work or light factory work did not affect serum creatine kinase activity.

The range and distribution of the normal serum creatine kinase values was not known at the beginning of this study. Okinaka *et al.* (1962) did not give figures but from their illustrations it may be seen that their upper limit of normal was around 5 arbitrary units, corresponding roughly to 4 IU. Dreyfus *et al.* (1960) reported normal values obtained by analyzing sera from 16 children and 33 adults. As can be seen from their original data, no normal or log normal distribution can be fitted to their data. In each group there are some unexpectedly high values. We suspect that these distorting figures may have been due to physical exercise prior to blood taking. As expected for theoretical reasons, the values of Schapira *et al.* (1960) are about 10 times higher than ours (see Methods).

For calculation of the normal range it was necessary to exclude a certain number of individuals. The first group which was deleted consisted of all pregnant females, newborns and children under the age of five (total 12). The second group included all children with muscular dystrophy presented in table 1 (total 23). Finally, all possible female carriers in type III families (assuming recessive sex-linked inheritance) were eliminated (total 98). There remained 305 individuals, 116 females and 189 males.

The distribution and cumulative frequency of serum creatine kinase values are shown in Fig. 1. Fig. 1a presents results on males and females, but separates those with a history of physical exercise from the rest. In Fig. 1b the data on females only are shown, again separated in a group of all results and a group excluding those with a positive history of exercise. Neither of the four groups shown in Fig. 1 fits a normal or a log normal distribution. Since our groups are too small for more complicated calculations it was decided to establish normal values by a graphic method. The cumulative frequencies of the different groups were plotted on log prob paper as shown in Fig. 7. The ranges of normal

TABLE 2. NORMAL VALUES OF SERUM CREATINE KINASE, BACKWARD REACTION, μ MOLES/MIN./1,000 ML. SERUM (37°C.), 50 PER CENT OF CUMULATIVE FREQUENCY AND RANGE (DETERMINED BY A GRAPHIC METHOD)

	N	50% mean	Upper limit of normal		
			5%	2%	1%
Total					
All sera	305	0.6	3.8	4.4	5.5
All males	189	0.8	3.8	4.7	6.2
All females	116	0.4	3.3	4.2	4.8
Excluding individuals with a history of physical exercise					
All sera	254	0.4	1.7	2.3	2.4
All males	148	0.5	1.8	2.5	2.8
All females	106	0.3	1.6	1.8	2.0

values taken from such graphs are presented in table 2. If the history in respect to physical exercise was taken into account, no differences between age groups were noted. 1.80 IU will be considered as the upper limit of normal in females and 2.50 IU as the upper limit of normal in males.

Serum Creatine Kinase Activity in Relatives of Type I Dystrophics

One family with two brothers affected by facioscapulohumeral dystrophy was investigated.

Family 12 (Wälchli): Cases 12 and 12a. No signs of the disease could be detected in

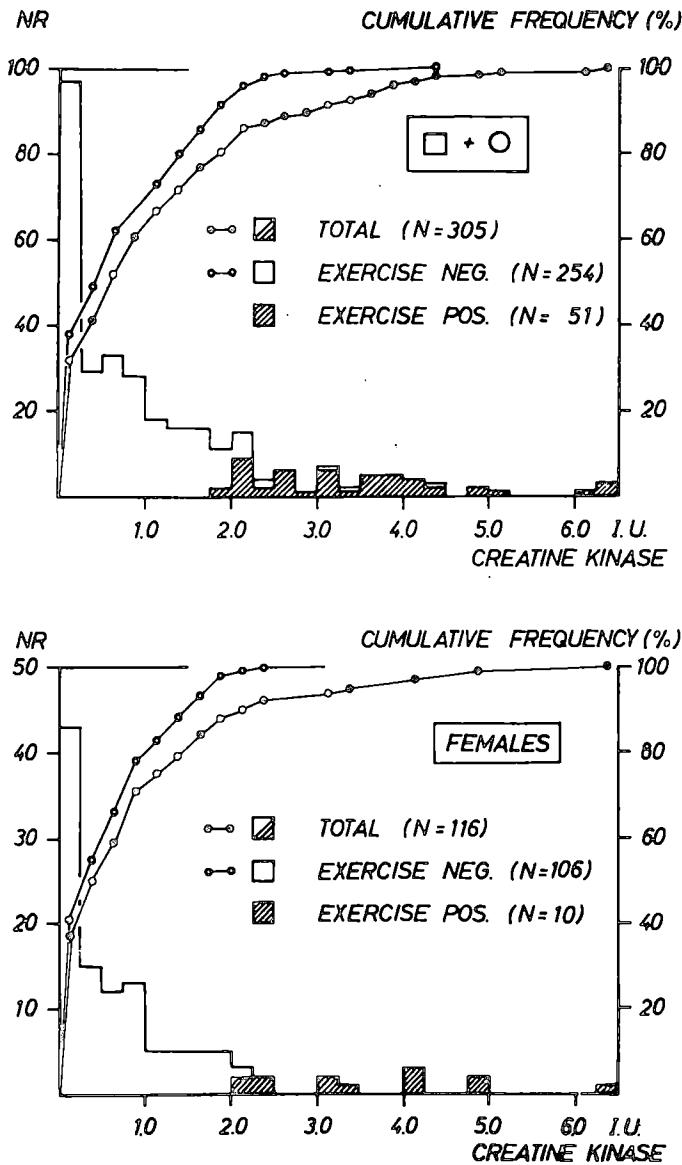


FIG. 1. Distribution of individual values and cumulative frequencies of the control groups.

21 relatives. Father 3.75 IU (had been working), mother 1.01 IU. Sisters of the father 0.0 and 1.16 IU. Sister of the mother 1.01 IU, and brothers of the mother 1.45 and 3.20 IU. More distant relatives: males 1.80, 1.66, 0.58, 3.75 (working), 0.85, 0.16 and 1.30 IU; females 2.46 (working), 1.01, 0.0, 0.73, 0.44, 1.60 and 1.01 IU.

Obviously more families must be investigated before conclusions can be drawn.

Serum Creatine Kinase Activity in Relatives of Type II Dystrophy

Five families of children with limb girdle type dystrophy were studied.

Family 4 (Luginbühl) : Only sister of the affected boy (Case 4) 0.58 IU. Mother 0.73 IU, father 0.58 IU. Maternal grandfather 1.31 IU and grandmother 0.0 IU. Paternal grandfather 0.0 IU. More distant relatives: males 1.60, 0.16, 1.60, 0.58, 1.00, 0.44, 0.16, 0.0, 0.16, 0.16, 0.0, 1.10, 0.0, 0.85, 0.0 (newborn) IU; females 2.02, 0.0, 0.58, 0.85, 0.44, 0.0, 0.85, 0.16, 0.87, 0.29, 0.80, 0.29, 1.36, 0.73, 4.20 (working), 4.50 (working) and 4.20 (working) IU.

Family 7 (Braun) : Only sister of the affected girl (Case 7) 2.05 IU. Father 1.08, mother 1.60 IU. Maternal grandmother 1.15 IU. Further relatives: males 1:15, 1:30, 1.75 (after working), 0.44 and 2.75 (after working) IU; females 0.22, 1.30 and 1.60 IU.

Family 10 (Kipfer) : Two sisters were affected, but only one was investigated (Case 10). A twin brother of one affected sister 1.31 IU. Mother 0.29, father 3.30 IU (after factory work). Maternal grandparents 0.0 IU. Further relatives: males 3.1 (after working), 0.44, 1.02, 0.85, 1.02, 0.29, 0.58, 1.16, 1.02, 0.73, 1.60, and six with 0.0 IU; females 0.24, 0.58, 0.44, 0.29, 1.44, 1.89 (after working), and seven with 0.0 IU.

Family 11 (Badertscher) : The only sister of the affected girl (Case 11) 1.31 IU. Father 1.31 IU, mother 0.0 IU. Paternal grandmother 0.15 IU. Maternal grandmother 3.25 IU (after working). Further relatives: males 0.0, 0.0, 0.0, 2.47 (after working), 0.44, 0.73 and 1.60 IU; females 0.29, 0.73, 0.03 (mongoloid trisomy), 0.15, 5.00 (after working) and 0.87 IU.

Family 13 (Kaiser) : One sister of the affected boy (Case 13) 0.50 IU; two sisters were not studied. The only brother 1.31 IU. Father 0.0 IU, mother 1.60 IU. Paternal grandparents 0.0 IU both. Maternal grandfather 0.0 IU. Relatives: males 0.29, 0.29, 0.73, 0.85, 0.50, 2.02, 1.01, 2.72 (after working), 1.44, 0.44, 0.73, 1.02 and six with 0.0 IU; females, three with 0.0, 0.85, 0.44, 0.29, 0.58, 0.85 and 0.10 IU.

Assuming recessive inheritance, abnormal heterozygote values should be observed in both parents if present. No such pattern was observed. In our experience identification of the carrier state in type II dystrophy is not possible by serum creatine kinase assays.

Duchenne Families: Classifications

For classification of the Duchenne families according to their formal and biochemical genetics it is necessary to define the carrier state.

Possible carriers (PC) : Females who might be carriers if recessive sex-linked inheritance is assumed, but who have normal creatine kinase activity.

Biochemical carriers (BC) : Females who genetically might be carriers if recessive sex-linked inheritance is assumed, and who have an elevated serum creatine kinase activity.

Genetical carriers (GC) : Mothers of affected children with at least one affected brother and an elevated serum creatine kinase activity.

Clinical carriers (CC) : Mothers of affected children with at least one affected brother, elevated serum creatine kinase activity and microsymptoms of muscular disease. Only one such female was encountered (Family 16).

Females who cannot be fitted into any of these categories but who have a

creatine kinase activity above 1.80 IU will be considered as "false positives." No "false negatives," (genetical carriers with normal creatine kinase activity) were encountered. If these definitions of the carrier state are used together with the pedigrees of the families four types of families can be distinguished.

1. Families with evidence for inheritance, *i.e.*, several affected individuals in different generations with the pattern of sex-linked recessive inheritance. Such typical pedigrees were present in families 5 and 16 only.

2. Families with no evidence for inheritance, and normal serum creatine kinase values in the mother and grandmother of the affected children. New mutations in the mother of the affected children may have been responsible for the disease in families 1, 2, 14, 17 and 19.

3. Families with no evidence for inheritance but with elevated serum creatine kinase values in the mothers and normal values in the grandmothers of the affected children. Occurrence of a mutation in the grandmother may have been responsible for the disease in families 3, 6, 18, 28 and 31.

4. Families with pseudosporadic cases of dystrophy, *i.e.*, with biochemical evidence for the carrier state in several generations but with affected males in one generation only.

Duchenne Families: Isolated Cases, Mutation Possibly in the Mother or Grandmother

In several families the biochemical data suggest the occurrence of a mutation in the mother of the affected boy. This was assumed if at least the mother and, if possible, sister of the mother of an isolated case had normal serum creatine kinase activity. Parts of the pedigrees of these families are shown in Fig. 2.

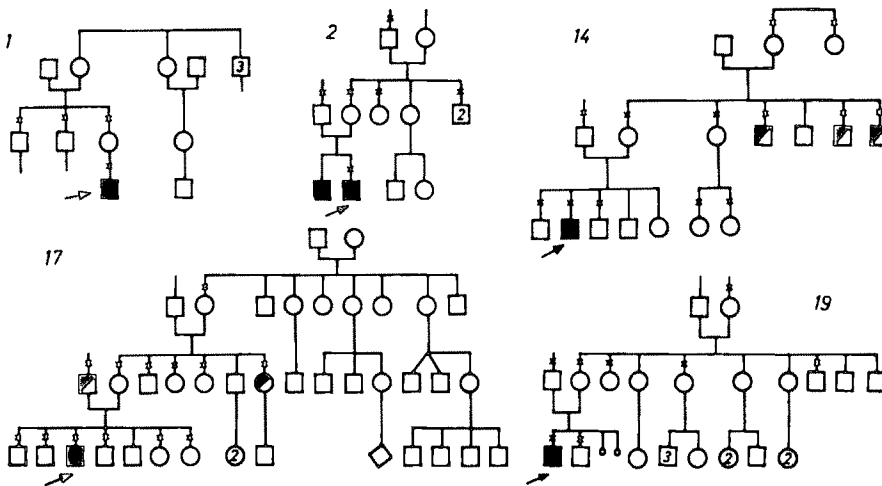


FIG. 2. Pedigrees of families 1, 2, 14, 17, and 19 (probably mutations in the mother). Open circles, females with creatine kinase < 1.80 IU; open squares, males with creatine kinase < 2.50 IU; circles black below diagonal, females with creatine kinase > 1.80 IU (clinical and biochemical carriers); circles black above diagonal, females with creatine kinase > 1.80 IU (false positives); squares with black above diagonal, males with creatine kinase > 2.50 IU; solid black squares, males with muscular dystrophy; arrow indicates proband; x on descent line indicates those examined for creatine kinase level.

Family 1 (Gasser): Mother of Case 1 0.29 IU (PC). Brothers of the mother 0.0 and 0.29 IU. Paternal male cousin 0.0 IU, and female cousin of the propositus 0.58 IU.

Family 2 (Gerber): Mother of Cases 2 and 2a 0.0 IU (PC). Sister of the mother 0.71 IU (PC), and brothers of the mother 0.88 and 0.58 IU. Father of the propositi 0.0 IU, and grandfather 2.20 IU.

Family 14 (Lanthemann): Brothers of Case 14 0.0 and 0.58 IU. Mother 1.46 and repeated 0.0 IU (PC). Sisters of the mother 0.30 IU (PC), her daughters 1.90 IU (aged 5) and 5.20 IU (aged 3). Brothers of the mother all returning from factory work 4.40, 3.40 and 2.75 IU. Maternal grandmother 0.0 IU (PC), her sister 1.30 IU (PC). Father of propositus 0.58 IU. Two paternal cousins returning from school 2.27 and 2.75 IU.

Family 17 (Küng): Brothers of Case 17 1.88, 2.05 and 0.73 IU. Sisters of propositus 1.60 (PC) and 0.0 IU (PC). Mother of propositus 0.44 IU (PC), her sisters 0.73 (PC), 1.02 (PC) and 2.30 (PC, false positive) IU. Brother of the mother 0.0 IU. Grandmother of propositus 0.73 IU (PC). Father of propositus returning from factory work 5.20 IU. Further paternal relatives: males 0.44, 0.0, 1.0 and 1.16 IU; females 0.45, 0.29, 0.85, 0.29, 1.60, 0.0 and 0.29 IU.

Family 19 (Bühler): Brother of propositus 0.73 IU. His mother 1.00, repeated 0.15 and 0.58 IU; His father 1.02 and repeated 0.20 IU. Sisters of the mother 1.00 IU (PC) and 0.0 IU (PC). Brother of the mother 1.47 IU. Grandmother of propositus 1.30 IU (PC).

A mutation in the grandmother was assumed if the mother of a sporadic case had an elevated serum creatine kinase activity, but her sisters and grandmother had normal values. Parts of the pedigrees of these families are shown in Fig. 3.

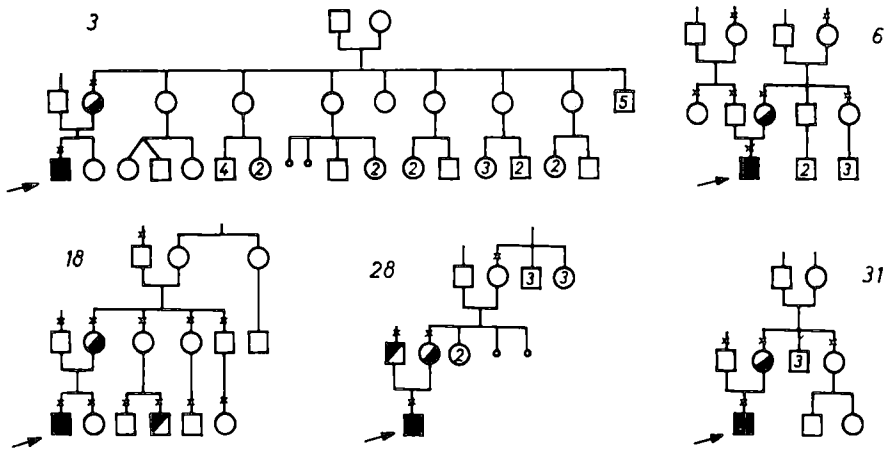


FIG. 3. Pedigrees of families 3, 6, 18, 28, and 31 (probably mutations in the grandmother). Legend see Fig. 2.

Family 3 (Brühlhard): Mother of Case 3 2.18 IU (BC).

Family 6 (Waibel): Mother of Case 6 1.88 IU (BC). Sister of the mother 1.31 IU (PC). Maternal grandmother 0.58 IU (PC). Father 0.58 IU, his sister 0.29 IU and mother 0.29 IU.

Family 18 (Alder): Sister of Case 18 0.0 (PC), his mother 2.05 IU (BC). Sisters of the mother 0.0 (BC) and 1.02 IU (PC). Their sons 0.03, 3.80 (after exercise) and 0.29 IU. Brother of the mother 0.0 IU, and his daughter 1.16 IU. Father of propositus 1.30 IU and grandfather 0.29 IU.

Family 28 (Nyffeler): Mother of Case 18 3.24 IU and repeated 2.30 IU (BC). Father after working 3.89 IU. Grandmother 1.50 IU and 0.81 IU (PC).

Family 31 (Walther): Mother of Case 31 3.24 IU (BC) and her sister 1.46 IU (PC). Father of propositus 1.30 IU.

Duchenne Families: Typical Pedigrees

Typical pedigrees for sex-linked recessive inheritance were present in two families only.

Family 5 (Suter): Fig. 4. Sister of propositus (Case 5) 6.80 IU (BC), his mother 2.46

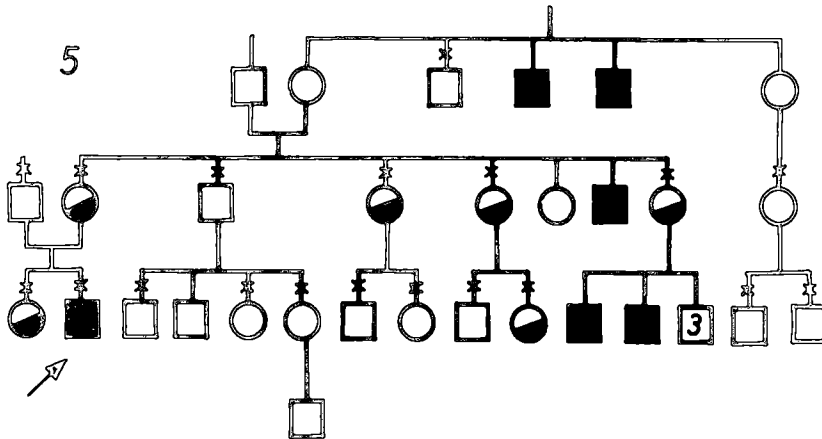


FIG. 4. Pedigree of family 5 (Suter). Typical pedigree for sex-linked recessive inheritance. Legend see Fig. 2.

IU (BC). First sister of the mother 3.41 IU (BC), her son 0.81 IU, and her daughter 0.73 IU (PC). Second sister of the mother 1.95 IU (BC), her son 0.0 IU and her daughter 2.17 IU (PC). Third sister of the mother 4.35 IU (GC) with two affected (Cases 5d and 5e) and three healthy children. Brother of the mother 0.15 IU, his son 0.0 and daughters 0.0 and 0.73 IU. One further brother of the mother died at the age of 12 from muscular dystrophy (Case 5c). He was not examined. Brother of the grandmother of propositus 0.27 IU; the affected brothers of the grandmother (Cases 5a and 5b) died from muscular dystrophy in early childhood and were not examined. Daughter of a sister of the grandmother (PC) 0.58 IU (PC), her two sons 0.50 and 0.60 IU. Sister of father 2.03 IU (false positive). A distant female relative of the mother 3.05 IU after working.

Family 16 (Schälin): Fig. 5. Sisters of propositus (Case 16) 0.15 (PC), 0.44 (PC), 0.73 (aged 3) and 2.30 IU (BC). Brother 5.8 (at birth), 2.6 (3 months old) and 6.6

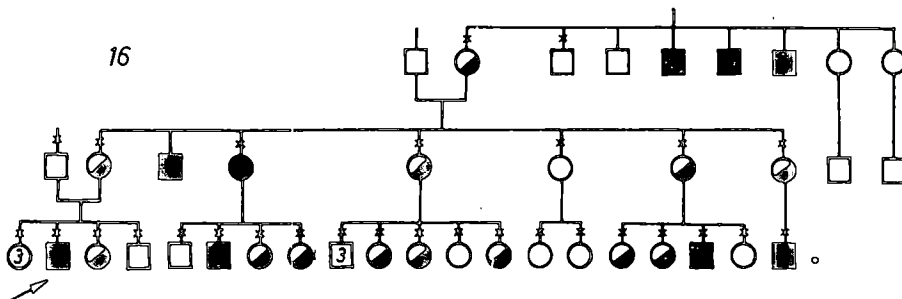


FIG. 5. Pedigree of family 16 (Schälin). Typical pedigree for sex-linked recessive inheritance. Legend see Fig. 2.

TABLE 3. SERUM CREATINE KINASE IN FEMALE RELATIVES AND MUSCULAR DYSTROPHY IN MALE RELATIVES OF AFFECTED CHILDREN. THE PROPOSITI, THEIR MOTHERS AND GRANDMOTHERS ARE NOT INCLUDED. GENERATIONS IN WHICH A MUTATION MIGHT HAVE OCCURRED ARE GIVEN IN PARENTHESIS

No. of family	5	16	5+16	9	15	22	26	30	9,15,22, 26,30	Total										
Serum creatine kinase (>1.8 IU vs. <1.8 IU)																				
Sisters of propositi	1	0	1	3	2	3	0	0	2	0	1	1	0	2	2	6	3	8	6	
Sisters of mothers of propositi	3	0	4	1	7	1	2	3	2	2	3	2	0	1	1	2	8	10	15	11
Sisters of grandmothers of propositi	0	0	0	0	0	0	(0	2)	0	0	0	0	0	0	0	0	0	0(2)	0	0(2)
Daughters of biochemically positive sisters of mothers (BC)	1	1	7	2	8	2	0	1	0	0	2	2	0	0	0	0	2	3	10	5
Total	5	1	12	6	17	7	2(0)	4(6)	4	2	6	5	1	1	3	4	16	16(18)	33	23(25)
Progressive muscular dystrophy (Muscular dystrophy vs. Healthy)																				
Brothers of propositi	0	0	0	1	0	1	0	0	0	2	0	3	0	0	0	0	0	5	0	6
Brothers of mothers of propositi	1	1	1	0	2	1	0	2	0	5	0	7	0	0	0	1	0	15	2	16
Brothers of grandmothers of propositi	2	1	3	2	5	3	(0	1)	0	0	(0	1)	(0	3)	(0	2)	0	0(7)	5	3(10)
Sons of biochemically positive sisters of mothers of propositi (BC)	2	5	3	4	5	9	0	1	0	3	0	6	0	0	0	0	0	10	5	19
Total	5	7	7	7	12	14	0(0)	3(4)	0	10	0(0)	16(17)	0(0)	0(3)	0(0)	3(2)	0	30(37)	12	44(54)
Sex ratio																				
♂ : ♀	12	8	14	20	26	28	4	9	9	9	17	16	3	4	3	11	36	49	62	77

IU (5 months old). Mother of propositus 1.9 and 10.3 IU postpartum (GC). Brother of mother (Case 16d) was not examined but is known to have died from typical muscular dystrophy (Case 3 of Sidler, 1944). First sister of mother (Case 16e) with signs of muscular disease (Case 4 of Sidler, 1944) 12.20 and repeated 2.60 IU (GC), her daughters 26.0 (BC) and 21.3 IU (BC), her healthy son 0.0 IU. Her affected son is Case 16f. Second sister of the mother of the propositus 21.0 IU (BC), her three healthy sons 0.58, 1.73 (after exercise) and 1.45 IU; her daughters 1.88 and repeated 1.80 IU (BC), 3.50 and repeated 1.82 IU (BC), 1.60 IU (PC), and 4.20 IU (BC). Third sister of the mother 0.27 and repeated 0.0 IU (PC), her daughters 0.0 (PC) and 6.90 IU (aged 3). Fourth sister of the mother 5.55, repeated 1.88 and 5.50 IU (GC), her affected son is Case 16g, her daughters 3.45 (BC), 3.45 (BC) and 10.0 IU (newborn). Fifth sister of the mother of the propositus 3.70, repeated 1.82 and 3.10 IU (GC), her affected son is Case 16h. Grandmother of the propositus 2.30 IU, her healthy brother 0.0 IU. The affected brothers, Cases 16a, 16b and 16c, died as adolescents from typical muscular dystrophy (Cases 5, 6 and 7 of Sidler, 1944). Father of propositus 0.45 IU. Further paternal relatives: males 0.0, 4.5 (after working), 0.25, 0.0, 0.0 and 0.29 IU; females 0.0, 0.0, 0.3, 1.16 and 2.30 (after working, false positive) IU.

The genetical and biochemical data of these two families are summarized in table 3. There are 13 males expected and 12 males observed with clinical evidence for muscular dystrophy. Seventeen female carriers were observed as compared to 12.0 expected. This discrepancy is hardly significant ($0.05 > p > 0.025$).

Duchenne Families: Pseudosporadic Cases

This last group of families seems to be genetically different from all other groups. Although the inheritance of the carrier state seems to follow the sex-linked recessive pattern through several generations, only sporadic cases of disease were encountered. The pedigrees of these families are shown in Fig. 6.

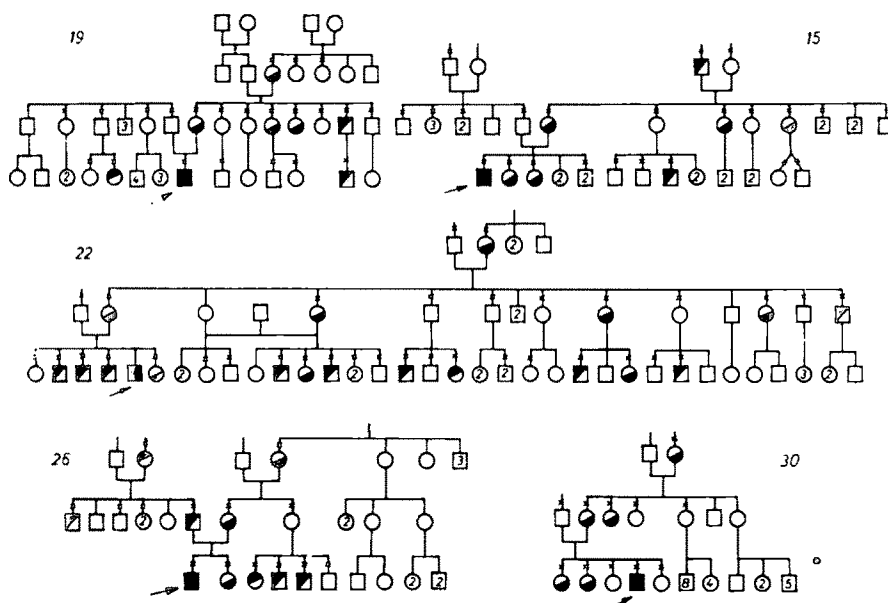


FIG. 6. Pedigrees of families 9, 15, 22, 26, and 30. Pseudosporadic cases. Legend see Fig. 2.

Family 9 (Graber): Mother of propositus (Case 9) 2.25 IU (BC). First sister of the mother 1.13 IU (PC), pregnant 10.0 IU; her son 13.42 IU (newborn). Second sister of the mother 1.15 IU (PC). Third sister of the mother 2.60 IU (BC), her son 0.0 and daughter 0.0 IU (PC). Fourth sister of the mother 8.00 IU (BC). Fifth sister of the mother 0.87 IU (PC). Brother of the mother 2.20 (working) and 1.15 IU; son of the first brother 2.20 IU (working). Grandmother of propositus 2.25 IU (BC), and her sisters 1.15 (PC) and 0.0 IU (PC). Father 0.0 IU. Sisters of the father 1.0 and 0.0 IU. Brother of the father 0.87 IU, and his daughters 0.87 IU and 4.10 IU (after exercise, false positive). Further paternal relatives: males 2.05 (working), 0.0 and 2.70 IU (working); female 0.0 IU.

Family 15 (Vuillemez): Sisters of Case 15 5.0 (BC), and 2.80 and repeated 2.72 IU (BC). Brothers of propositus 1.73 and 8.70 IU (newborn). First sister of mother 1.60 IU (PC), her sons 1.90, 2.45 and 4.35 IU (all after working). Second sister of the mother 2.80 IU (BC). Third sister of the mother 0.44 IU (PC). Fourth sister of the mother 3.70 and repeated 1.82 IU (BC), her son 6.23 IU (aged 3), and her daughter 1.36 IU (aged 3). Brothers of the mother 1.16, 1.36 and 2.18 IU (after working). Father of propositus 0.58 IU, his brothers 2.10 (after working), 1.10 and 0.70 IU, his sisters 0.50, 1.80 (pregnant) and 2.70 (pregnant) IU. Paternal grandfather 0.58 IU.

Family 22 (Flückiger): Sisters of propositus (Case 22) 1.60 (PC) and 11.00 IU (BC). Brothers of propositus 2.60 (after working) and 3.70 IU (after working). Mother of propositus 2.59 and repeated 1.80 IU (BC). Son of first sister of the mother 0.0 IU, her daughter 0.0 IU. Second sister of mother 3.25 IU (BC), her sons 6.25 IU (working), 2.72 and 2.11 IU (after exercise), her daughters 1.87 (BC), 1.46 (PC) and 1.62 IU (PC). Third sister of the mother 0.0 IU (PC), her daughter 0.0 IU (PC). Fourth sister of the mother 1.84 IU (BC), her son 6.50 IU (working) and her daughter 1.95 IU (BC). Fifth sister of the mother 0.80 IU (PC), her sons 0.40 and 3.20 IU (working). Sixth sister of the mother 1.95 IU (BC). First brother of the mother of the propositus 1.50 IU, his sons 3.60 (working) and 1.60 IU. Second brother of the mother 0.97 IU, his sons 0.32 and 0.0 IU. Sixth brother of the mother 1.87 IU. Seventh brother of the mother 3.89 IU (working). Grandmother 3.57 IU (BC) and grandfather 1.14 IU. Father 1.60 IU.

Family 26 (Weisskopf): Sister of propositus (Case 26) 2.11 IU (BC). Mother of propositus 5.80 IU (BC). Sister of mother 1.62 IU (PC), her daughter 1.94 IU (PC) and her sons 3.90, 3.72 and 2.20 IU, all returning from work. Maternal grandmother 2.27 IU (BC). Father 3.20 IU (after working), his brothers 4.10, and 1.94 IU (both returning from factory work), and sisters 1.46 and 0.50 IU. Paternal grandmother 1.94 IU.

Family 30 (Amrein): Sisters of propositus (Case 30) 2.92 (BC), 5.35 (BC), 1.58 (newborn) and 1.14 IU (PC). Mother of propositus 2.27 IU (BC). Sisters of the mother 2.27 (BC), 3.73 (BC), 1.62 (PC) and 0.0 IU (PC). Grandmother 2.92 IU (BC). Father 1.78 IU (after working).

The data on these families with sporadic cases of progressive muscular dystrophy are also summarized in table 3. Sixteen carriers were observed and 16 expected. This is in agreement with the assumption of sex-linked recessive inheritance. If the number of the affected males in the carrier generations are counted a discrepancy between the expected and the observed number is found. Although 15 males should be affected, none revealed any clinical signs of muscular dystrophy. It seems impossible that such a discrepancy might occur by chance only.

Identification of Carriers in the Duchenne Type: Quantitative Interpretation

The results of serum creatine kinase assays in normals, carriers and

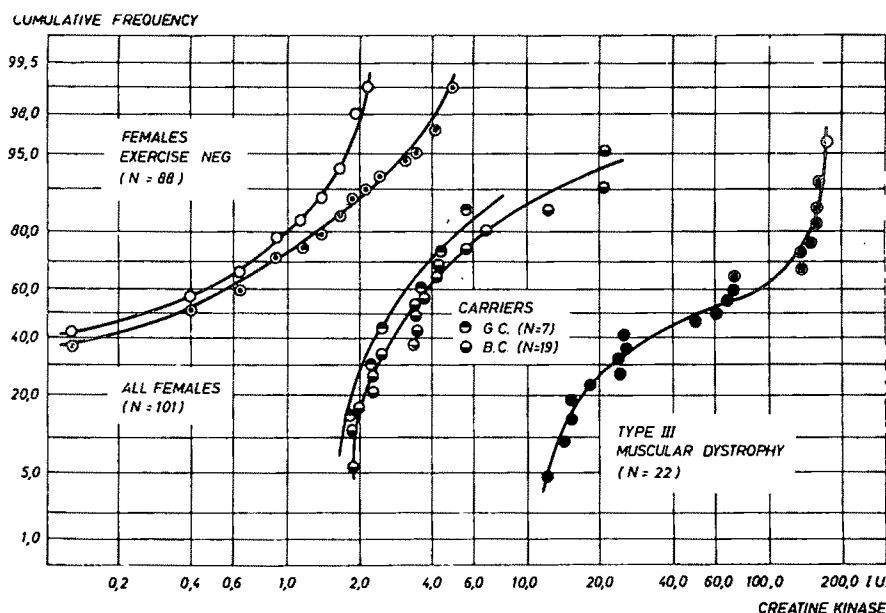


FIG. 7. Cumulative frequencies of females without history of exercise (open circles), of all females examined (dotted circles), of genetical carriers (circles black above), and of biochemical carriers, (circles black below), and of patients with type III muscular dystrophy (solid circles).

dystrophics become even more convincing if they are considered from a quantitative viewpoint. In Fig. 7 the data of the serum creatine kinase values of the genetical carriers of families 5 and 16 are plotted separately from those of the biochemical carriers (families 9, 15, 22, 26, 30). The two curves obtained are identical. Identity of the values and their distributions suggests biological homogeneity of the two groups. If the carriers are compared to the normal population and to the dystrophics, further interesting observations emerge. First, the carriers take up an intermediate position between normals and dystrophics. There is some overlap between creatine kinase values of normals and carriers and between carriers and dystrophics. Second, the shape of the curve of the carriers is similar to the shape of the dystrophics suggesting a similar type of distribution. It differs markedly from the form of the curve obtained from the normal population.

The Discrepancy Between Expected and Observed Number of Affected Males in Families with Biochemical Evidence for Inheritance

It has previously been noted by several investigators that there exists a discrepancy between the expected and observed number of affected males in families with isolated cases. In the sibships containing more than one male, with no cases in previous generations, the incidence of affected males was 33 per cent (Walton, 1955), 22 per cent (Stevenson, 1955), and 18 per cent (Stephens and Tyler, 1951) instead of the expected 50 per cent. This discrep-

inheritance is assumed. In the remaining five families (9, 15, 22, 26 and 30) the female carrier number is again in agreement with sex-linked recessive inheritance but the number of affected males in the carrier generations is too low (0 observed and 15 expected).

There are several mechanisms which might explain this discrepancy. First, the gene may have a lethal effect early in pregnancy. If this were the case the sex ratio should be disturbed. In families 5 and 16 the ratio of males to females is 26 to 28, in all families 62 to 77. Early fetal death seems therefore an improbable explanation.

Second, it is at present assumed that the gene for type III dystrophy has a high penetrance. Nevertheless, if the carrier state cannot be recognized, the existence of subclinical disease in males cannot be excluded. Let us assume that the gene might lead either to the full clinical manifestations of the disease ("muscular dystrophy") or to no clinical signs at all ("latent affection"). In this case one would expect elevated creatine kinase values in the males with "latent disease." To test this hypothesis families 15 and 22 were selected since they were the only ones with enough males investigated. In Fig. 8 all males of these two families are classified in two groups, one group corresponding to the individuals who could be latently affected and one group corresponding to those which could not be affected if sex-linked recessive inheritance is assumed. In both categories, those with and those without a history of exercise, the individuals with possible latent disease show higher values. Using the X-test of van der Waerden and Nievergelt (1956) the difference is highly significant ($p < 0.01$) for the group without a history of exercise.

Third, our observations might also be explained by assuming autosomal inheritance with sex-limited manifestation of the disease. This hypothesis, nevertheless, is in contradiction to the observations that the few fertile male dystrophics observed so far did not have affected children (Walton, 1955), and that linkage with color blindness occurred in a family investigated by Philip and Walton (1956).

DISCUSSION

The investigations of Stevenson (1953) and Walton (1955, 1956) and the statistical analyses by Chung and Morton (1959) have established the existence of at least three major types of progressive muscular dystrophy which differ in their inheritance, clinical manifestations and course. In several studies it has been shown that serum enzymes, particularly serum aldolase, are markedly elevated in type III dystrophy though minor increases are found in the other types too. Recent investigations (Aebi *et al.*, 1961) have established that serum creatine kinase is a better discriminant than other serum enzymes. In children with type III dystrophy the average of serum aldolase, glutamic pyruvic and glutamic oxalacetic transaminase and lactic dehydrogenase are about 15-20 standard deviations above the mean of the normal range. In the same group of patients serum creatine kinase values were 90 standard deviations above the mean of healthy individuals. This observation suggests that the increase in serum creatine kinase may be on a different level than the elevation of other enzymes.

A number of investigators have attempted to identify the carrier state by serum enzyme assays. Entirely negative results were obtained by the use of serum lactic dehydrogenase (Brugsch, Brockmann-Rohne and Fromm, 1960; Soltan, and Blanchaer 1959), glutamic pyruvic transaminase (Brugsch *et al.*, 1960), and glutamic oxalacetic transaminase (Brugsch *et al.*, 1960; Leyburn, Thomson and Walton 1961). Using serum aldolase activity the results are controversial. Soltan and Blanchaer (1959), Evans and Baker (1957), and Leyburn *et al.*, (1961) found no evidence for increased aldolase activity in relatives of patients. Dreyfus and associates (Dreyfus *et al.*, 1960; Schapira *et al.*, 1960) observed elevated aldolase values in a small number of mothers of children with Duchenne dystrophy. Chung, Morton and Peters (1960) found slightly higher average serum aldolase values in the carrier group than in the normal population. Some of the sisters and mothers had markedly increased enzyme activities and the authors felt that they were able to detect some of the carriers of the disease.

Two groups of workers have tried to apply serum creatine kinase studies to the carrier problem. Okinaka *et al.* (1961) could not find elevated values in the parents of five of their children but observed abnormal levels in some sisters of the patients. The French workers (Dreyfus *et al.*, 1960; Schapira *et al.*, 1960) found high activities in the sera of some of the mothers of their patients. They believe that identification of all carriers is possible if aldolase and creatine kinase assays are combined with a measurement of the circulation time (Dreyfus and Schapira, 1962). In both studies no effect of physical exercise on serum enzymes is mentioned.

One of our most difficult problems has been to establish normal values of serum creatine kinase activity, first, because physical exercise may lead to markedly increased values and second, because normal values are distributed in a very unusual way. A graphic method had to be chosen to establish the upper limit of normal. For this purpose it seemed particularly useful to use log prob paper for the plotting of the results. Log prob paper is frequently used in pharmacology in the evaluation of dose effect relationships (Litchfield and Wilcoxon, 1949). As far as we are aware, it has not been used in human genetics although it offers a number of advantages. Not only does the type of distribution of the values in the different groups not matter, but its use allows immediate evaluation of the probability that an individual value falls into a particular group. The five most important groups, normal females without a history of exercise, all females, genetical and biochemical carriers and dystrophics are plotted on log prob paper in Fig. 7. The carrier group takes an intermediate position between the normals and the dystrophics. The shape of their curve is similar to the one of the dystrophics but different from the curve of the normals. Both observations are in agreement with the assumption that we are dealing with a true carrier state, showing qualitatively a similar biochemical defect (same shape of curve as dystrophics) and lying quantitatively intermediate between dystrophics and normals.

From Fig. 7 the chances that an individual result will fall within a particular group can easily be predicted. If exercise is excluded, 2 per cent of normal females will have values above 2 IU and 20 per cent of the carriers will be

below this limit. If determinations are repeated several times the probability of missing a carrier or misclassifying a normal person will become very low. The method therefore permits individual diagnosis or exclusion of the carrier state with a considerable degree of certainty. The method should be helpful in genetic counseling. Since the frequency of carriers of the sex-linked recessive form of muscular dystrophy is about 16 per thousand births, the method may even prove suitable for the screening of selected populations.

Biochemical expressivity of the gene in carriers is high. As in other heterozygote states there is some overlap between normals and carriers and between carriers and affected individuals. This leads one to suspect that some of the carriers may show microsymptoms of the disease. Kryschora and Abowjan (1934) have observed dysplastic symptoms (kyphoscoliosis, pseudohypertrophy, facial weakness) in some clinical carriers in Duchenne families. Chung, Morton and Peters (1960) observed typical symptoms of mild limb girdle dystrophy with aldolasemia in a 50 year old mother of a boy with Duchenne type dystrophy. We have observed an additional similar case. Patient 16 f was first reported by Sidler (1944) as suffering from mild dystrophy of the limb girdle type. Her clinical manifestations are still the same 17 years later although she has serum creatine kinase values of 16 IU. It is important to note that in all these cases the symptomatic carrier resembles more a mild limb girdle type dystrophy than a Duchenne form. Chung, Morton and Peters (1960) suggested that some of the sporadic limb girdle cases might be carriers of the type III gene.

A relatively large number of the Duchenne type dystrophies occur sporadically. This has been explained by assuming a high mutation rate in the order of 5.4 to 7.2×10^{-5} (Stevenson, 1955), 4.3×10^{-5} (Walton, 1955), and 9.5×10^{-5} (Stephens and Tyler, 1951). In our series five of 17 cases may have been due to a mutation in the mother and an additional five due to a mutation in the grandmother. In the remaining seven families a reasonable number of possible carriers had creatine kinase values above 1.8 IU. In all seven families the distribution of these carriers was in accordance with a sex-linked recessive gene. Nevertheless, when these families were analyzed two entirely different groups were noted. In two families (5 and 16) the number of affected to unaffected males approached the theoretical 1:1 ratio. In the remaining five families about 15 affected males were expected in the female carrier generations but none was observed. This discrepancy might be explained by two alternative hypothesis. Either the gene is inherited in an autosomal way and its expression is sex-limited or there are clinically two types of the disease, one with complete penetrance and one with very low penetrance.

If the second hypothesis is true the following four types of affected individuals are expected. First, patients with typical Duchenne's disease and high serum creatine kinase. Second, males with latent dystrophy (no clinical signs), but possibly elevated creatine kinase values. Third, female carriers with no clinical signs but an elevated serum creatine kinase. Fourth, female clinical carriers with an elevated serum creatine kinase and mild and atypical symptoms of muscular dystrophy of type II.

We favor the hypothesis that muscular dystrophy in males occurs in a clinically apparent and a clinically latent form since it would explain the

occurrence of the disease in females. Recent observations have shown that about 10 per cent of all Duchenne cases occur in girls (Lamy and de Grouchy, 1954; Becker, 1957; Klopfer and Talley, 1958; Blyth and Pugh, 1959). Several attempts have been made to explain this puzzling feature. Walton (1956) reported a Duchenne type dystrophy in a female with Turner's syndrome, *i.e.*, male chromosomal sex. Such a combination must be extremely rare and cannot explain the frequent occurrence of Duchenne's disease in girls. Walton (1955) pointed out that the affection might occur in girls by the mating of a female carrier with a male upon whose X-chromosome a mutation had occurred. Such an explanation would only be satisfactory if it is taken in conjunction with the suggestion of Haldane (1956) that the mutation rate might be much higher in the male than in the female. This suggestion has been disproved by Cheeseman, Kilpatrick, Stevenson, and Smith (1958). Finally, it has been suggested that in families with affected girls the disease might be inherited in an autosomal recessive way (Klopfer and Talley, 1958; Blyth and Pugh, 1959; Becker, 1957; Dubowitz, 1960; Lamy and de Grouchy, 1954).

As long as it is not possible to detect female carriers it will not be possible to decide which one of these explanations is true. Our observations point to an alternative hypothesis. They suggest the existence of two types of dystrophy in males, one being clinically latent and one leading to typical Duchenne's disease. Mating between a male who has the disease in the latent form (reduced penetrance) with a carrier female would explain the occasional occurrence of the affection in girls. As far as we are aware there are no arguments against such an assumption in the literature on formal genetics of the Duchenne type. It should be mentioned that the estimations of the mutation rates may not be valid if our assumptions are true. Obviously it will be necessary to study a much larger number of families to substantiate or contradict this hypothesis.

SUMMARY

1. Serum creatine kinase was determined in 438 relatives of one patient with facioscapulohumeral, five patients with limb girdle and 16 patients with Duchenne type muscular dystrophy. Enzyme activity was markedly elevated in children with type III dystrophy but only slightly elevated or normal in the other two types of muscular disease. In healthy persons serum creatine kinase activity rises slightly following physical exercise.

2. There was no evidence for abnormally high serum creatine kinase values in relatives of children with facioscapulohumeral and limb-girdle type dystrophy.

3. In the Duchenne type families the expected number of female carriers had elevated serum creatine kinase values. There was some overlap between normals and carriers and between carriers and dystrophics.

4. Ten of 17 cases of Duchenne dystrophy were probably due to new mutations occurring either in the mother or in the grandmother of the affected boy. Two families showed the expected ratio of affected to unaffected males. In the remaining five families there were no affected males if the propositi were excluded, although 16 should have been expected in the carrier generations investigated assuming sex-linked recessive inheritance and high penetrance in affected individuals.

5. From our data it seems probable that there exist two forms of the Duchenne type dystrophy, one with complete penetrance and one with low penetrance in affected males. This hypothesis would also explain the occurrence of the disease in girls.

6. A genetical female carrier with mild and atypical symptoms of muscular dystrophy is reported.

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"Mixed" Gonadal Dysgenesis: A Variety of Hermaphroditism

ARTHUR R. SOHVAL

*Endocrine Research Laboratory and Clinic of the Department of Medicine,
The Mount Sinai Hospital, New York, New York*

PERUSAL of the results of cytogenetic studies in individuals with sex anomalies (Sohval, 1961; 1963a) suggested the existence of a special type of intersex which has not yet been classified definitively. These individuals appear to have sufficient clinical and anatomic features in common to warrant their consideration as a distinct variety of hermaphroditism. They may present as phenotypic males or females but the nuclear sex in all cases studied to date is chromatin-negative. As in all intersexes, the reproductive system exhibits a mixture of male and female elements. These include an enlarged clitoris or phallus with urethral opening at its base, a vagina, uterus and Fallopian tubes as well as an intra-abdominal testis.

The nosologic difficulty stems from the state of the gonads. Situated intra-abdominally in the normal position of ovaries, these consist of a rudimentary or vestigial "streak" gonad on one side and a testis on the other. Since the former is identifiable as neither testis nor ovary, such cases cannot be properly categorized on the basis of gonadal histology as male pseudohermaphrodites (bilateral testes), female pseudohermaphrodites (bilateral ovaries) or true hermaphrodites (presence of both testicular and ovarian tissue).

Five such cases are now to be found in the recent literature. These are the patients of Ferguson-Smith and Johnston (1960), Bloise *et al.* (1960), Conen *et al.* (1961), Schuster and Motulsky (1962a; 1962b) and Willemse, van Brink and Los (1962). The latter three are phenotypic female adults and the first two are children raised as boys. Karyotype analysis disclosed the presence of cells with an XO sex-chromosome complement in each case. In the two male children these cells were the only type demonstrated (only bone marrow cells were cultured). However, XO/XY sex mosaicism was encountered in cultured leukocytes of peripheral blood in two of the females (Conen *et al.* 1961; Willemse *et al.* 1962) while a triple mosaic XO/XY/XX, was found in cultures of skin and peripheral blood of the third female (Schuster and Motulsky, 1962a; 1962b).

There are four other patients who seem to qualify for inclusion in this group although complete clinicopathologic data are not supplied. They are among nine individuals described as male pseudohermaphrodites by Alexander and Ferguson-Smith (1961). Each had an XY sex-chromosome complex in their cultured bone marrow cells.

The term "mixed" gonadal dysgenesis is offered as a tentative designation for these examples of intersexuality which are usually not classified decisively in the literature. The term "mixed" refers to the coexistence of different forms

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of defective gonadogenesis in the same individual. The gonadal dysgenetic process is more marked than that encountered in classical true or false hermaphroditism but is less severe than that which characterizes the bilateral "streak" gonads of "pure" gonadal dysgenesis and Turner's syndrome. The overall condition of the gonads in these cases apparently represents an intermediate form between "pure" gonadal dysgenesis on one hand and male pseudohermaphroditism or true hermaphroditism on the other.

The fact that this condition has been described in five cases and alluded to in four others within the past two years suggests that intersexes with a "streak" gonad on one side and an intra-abdominal testis on the other are not too rare. The general lack of such cases in the prior literature probably reflects a common unawareness of the inconspicuous "streak" gonad as a pathologic entity before its description by Wilkins and Fleischmann (1944).

The question may be raised as to whether or not an intersex patient with a rudimentary, undifferentiated gonad on one side and a testis on the other should be regarded as an instance of male pseudohermaphroditism, or possibly as a variant of it (Alexander and Ferguson-Smith, 1961). However, the accumulated results of chromosome analysis in persons with anomalous sex development strongly suggest the existence of a clear distinction between intersexes who have classical male pseudohermaphroditism (with bilateral testes) and those with "mixed" gonadal dysgenesis. Whereas an XY sex karyotype is almost invariable in the former, the XO complex seems to predominate in the latter, occurring alone or more probably as one component of a mosaic. In the instances where an XO constitution was not found (Alexander and Ferguson-Smith, 1961) the possibility of sex mosaicism with an undetected XO stem-line of cells cannot be excluded.

The demonstration of significant numbers of XO cells, existing alone or as part of a sex mosaic, suggests that chromosomal aberrations play a major role in the pathogenesis of "mixed" gonadal dysgenesis. The presence of two different kinds of gonads in the same individual is readily explainable on the basis of sex-chromosome mosaicism, a finding already documented in three of the patients. In the cases under discussion, the existence or predominance of the XO sex complement in one gonadal anlage with a different sex karyotype (*i.e.*, XY) dominant in the other would account for the markedly dissimilar gonadal anatomy ("streak" gonad on one side and testis on the other). An alternative, although less likely, explanation is the possibility that other teratogenic factors of a chance nature may be operative locally and unilaterally during very early gonadogenesis.

While the evidence suggests that this atypical form of hermaphroditism with "mixed" gonadal dysgenesis is due to sex-chromosome mosaicism, especially of the XO/XY type, the converse does not always hold. In other words, XO/XY mosaicism does not necessarily result in this variety of intersex. This is indicated by the fact that XO/XY mosaics have also been reported in nine other chromatin-negative individuals with various kinds of sex anomalies. With one exception all are phenotypic females.

Included in this miscellaneous group of persons with XO/XY mosaicism and aberrant sex development is the patient of Hirschhorn, Decker and Cooper

(1960a; 1960b) who may be a true hermaphrodite although the gonadal histology is not conclusive. Each of the two intersexes studied by Miller, Breg and Jailer (1960) had only one gonad. This was intra-abdominal in location and was composed predominantly of testicular tissue in which a seminoma had developed. The case of Judge *et al.* (1962) appears to be an instance of "pure" gonadal dysgenesis although histologic examination was performed in only one of the two very small gonads. The two individuals studied by Jacobs *et al.* (1961) have Turner's syndrome. A patient observed by Miller, Breg and Schmickel (unpublished) has a variant form of Turner's syndrome. The adult male reported by de la Chapelle and Hortling (1962) is a unilateral cryptorchid with hypospadias. Since abdominal exploration was not performed the character of the internal genitalia and other gonad, if any, is not known. In the patient studied by Blank, Bishop and Caley (1960) there was a small intra-abdominal gonad on one side but its histology was not determined. No gonad could be identified on the opposite side.*

From these observations it is evident that XO/XY mosaicism is indeed associated with diverse types of sex anomalies. As in most forms of defective gonadogenesis the associated clinical and anatomic features depend more upon the extent and nature of the fetal testicular secretory failure than upon the precise sex-chromosome constitution, a point recently stressed by Dewhurst (1962).

Recognition of intersexes with "mixed" gonadal dysgenesis as a distinct variety of hermaphroditism should help in the evaluation and classification of atypical forms of gonadal dysgenesis with anomalous sexual development. Moreover, insistence on rigid diagnostic criteria based on gonadal histology will serve to render more meaningful the interpretation of associated sex-chromosome findings.

A detailed discussion of this type of hermaphroditism, its variant forms and its relationship to gonadal neoplasm is presented elsewhere (Sohval, 1963b).

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Control Gene Mutation as a Possible Explanation of Certain Haptoglobin Phenotypes

W. CAREY PARKER AND ALEXANDER G. BEARN

The Rockefeller Institute, New York, New York

OF RAPIDLY developing interest in genetics is the functional duality of mechanisms for structure and control in living organisms, by which discrete elements of the genetic constitution appear to be involved not only in the specificity of natural products but also in the regulation of their production. The understanding of these mechanisms of control at the interwoven levels of both biochemistry and genetics has reached its most extensive expression in the elaborate network of hypothesis and experimental verification developed by Jacob and Monod (1961) for the β -galactosidase system in *Escherichia coli*.

In the field of human genetics, numerous examples of hereditary variations in the elements of structure and control may be found in the abnormalities of hemoglobin and the plasma proteins. The classical example of mutation in a structural gene is the biochemical modification determined by Ingram (1956) in sickle-cell hemoglobin; Neel (1961), Ceppellini (1961), and Motulsky (1962) have analyzed certain hemoglobin diseases in terms of mutations in a control system, and the present authors have recently applied the concepts of genetic regulatory mechanisms in bacteria to the hemoglobin and glucose-6-phosphate dehydrogenase systems in man, and to the problem of antibody synthesis (Parker and Bearn, 1963). Among the plasma proteins, the hereditary deficiencies of albumin (Bennhold, Peters and Roth, 1954), ceruloplasmin (Bearn, 1960), transferrin (Heilmeyer *et al.*, 1961), α -lipoprotein (Fredrickson *et al.*, 1961), and β -lipoprotein (Salt *et al.*, 1960) indicate a possible abnormality in the control element. Genetic variations in the structural element include the variants of albumin (Knedel, 1957), transferrin (Parker and Bearn, 1961), the Gc system (Hirschfeld, 1959; Cleve and Bearn, 1961) and the Gm system (Grubb and Laurell, 1956). It is the purpose of this report to suggest that certain unusual phenotypes in the haptoglobin system may represent genetic alterations in the control system for this protein.

HAPTOGLOBIN PHENOTYPES OF HUMAN SERUM

The serum haptoglobin phenotypes are determined by two alleles, Hp^1 and Hp^2 , which can combine to form the three common phenotypes Hp 1-1, Hp 2-1 and Hp 2-2 as distinguished by conventional starch gel electrophoresis (Smithies, 1955; 1959). In addition to the three common phenotypes, Connell and Smithies (1959), Giblett (1959a), and Blumberg, Allison, and Garry (1959) have observed a phenotypic variant in Negro populations which has been labelled Hp 2-1 modified (Hp 2-1 (mod) or Hp 2-1M) because of its resemblance to

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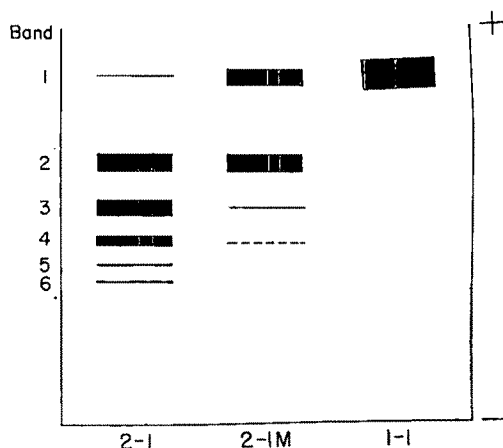


FIG. 1. Diagram of starch gel electrophoretic pattern illustrating the migration of the haptoglobin phenotypes Hp 2-1, Hp 2-1M, and Hp 1-1.

Hp 2-1. The variant phenotype has also been described by Kirk and Lai (1961) in a Malay and a Tamil individual from Malaya. Fig. 1 illustrates the observed variation between the Hp 2-1 and Hp 2-1M phenotypes as determined by starch gel electrophoresis. This variation consists of an increased proportion of the two fastest-moving haptoglobin components in the Hp 2-1M type, together with a greatly decreased amount of the slower-moving components. Various authors have noted that in certain instances the slower-moving haptoglobin bands of the Hp 2-1M phenotype may show variations in intensity which sometimes make this phenotype difficult to distinguish from the normal Hp 2-1 phenotype (Barnicot, Garlick and Roberts, 1960; Giblett, 1963). An additional phenotype, Hp 0, characterized by the absence of haptoglobin as detected by the benzidine stain in starch gel electrophoresis, has been reported by several authors in individuals of many population groups. In cases where depletion of haptoglobin as a result of various hemolytic disorders can be excluded, the absence of haptoglobin (ahaptoglobinemia) has been attributed to genetic origin. Other phenotypic variants of haptoglobin have also been described, but the principal emphasis in the present discussion is upon the Hp 2-1M and Hp 0 phenotypes.

Since the Hp 2-1M phenotype and hereditary ahaptoglobinemia are frequently observed in a single pedigree in Negro populations, their inheritance has been of considerable interest. Giblett and Steinberg (1960), in a careful and extensive study of 92 Negro families, presented evidence for the existence of an allele Hp^{2m} at the haptoglobin locus. Their data excluded an interpretation of ahaptoglobinemia as caused either by a recessive allele (Hp^0) or by a dominant suppressor with complete penetrance. However, it appears somewhat difficult to explain all of the data in terms of the Hp^{2m} allele of Giblett and Steinberg. These authors postulated that the genotype Hp^1/Hp^{2m} could be represented by either of two phenotypes, Hp 2-1M or Hp 0. On this basis it was necessary to assume that the Hp 0 child of a Hp 1-1 x Hp 1-1 mating was

extramarital. Subsequent studies by Steinberg *et al.* (1961) in Chinese and Indian populations have revealed two other children whose phenotypes are inconsistent with this hypothesis; in each case an ahaptoglobinemic child was the offspring of Hp 2-2 parents. The Hp 2-1M offspring of a Hp 1-1 x Hp 2-1 mating reported by Barnicot *et al.* (1960) is also inconsistent with the hypothesis. To explain certain pedigrees it was also necessary for Giblett and Steinberg to make the additional assumption that ahaptoglobinemia could result from the genotype Hp^1/Hp^2 if the Hp^1/Hp^{2m} genotype never resulted in a Hp 2-1 phenotype. Finally, it was not possible for Giblett and Steinberg to determine the phenotypes expressed by their genotypes Hp^2/Hp^{2m} and Hp^{2m}/Hp^{2m} .

CONTROL GENES AND STRUCTURAL GENES IN THE SYNTHESIS OF
HAPTOGLOBIN. ABSOLUTE LINKAGE OF THE CONTROL
AND STRUCTURAL GENE LOCI

In view of the difficulty in accounting for all of the observed data on the basis of the Hp^{2m} allele hypothesis, it is of interest to determine whether an alternate hypothesis can be established. If, instead of analyzing haptoglobin inheritance in terms of variation in structural alleles, the unusual haptoglobin phenotypes are considered in terms of an alteration in a control gene, it is possible to construct an alternate interpretation which appears to account for all of the data presently available on the inheritance of the Hp 2-1M and Hp 0 phenotypes. According to the accepted theory of haptoglobin polymerization (Allison, 1959), the observed pattern for the Hp 2-1M phenotype (Fig. 1) can be considered as arising from a reduced formation of the Hp 2 gene product, such that less of the Hp 2 monomeric unit is available for combination with the Hp 1 unit. In this case, the Hp 2 unit will be consumed primarily in the formation of the most rapidly moving component (Fig. 1, band 2) in the position of the slow-moving haptoglobins in the starch gel, with only minor formation of the higher polymers (bands 3-6) present in the normal Hp 2-1 phenotype; the excess Hp 1 unit will appear as an increased amount of haptoglobin in the Hp 1-1 position (band 1).

Smithies, Connell, and Dixon (1962a, 1962b) have reported that haptoglobin is dissociable into α and β polypeptide chains, that the genetic determinants of haptoglobin phenotype reside in the α unit, and that the Hp 2 unit (α^2) represents a fusion of two Hp 1 (α^1) units into a single molecule. On the basis of these results, it is possible to construct a model for the polymerization of the β and α haptoglobin chains which is consistent with the observed phenotypes. The molecular structure of the components of the Hp 1-1 and Hp 2-1 phenotypes is given in detail in table 1; the method of quantitation of the individual haptoglobin components is described in the following section. By this model, the Hp 2-1M phenotype may be considered to arise from a decreased synthesis of α^2 units. It is apparent by such a model that the Hp 2-2 phenotype would consist of polymer components of molecular structure $(\beta\alpha^2)_n$ where $n = 3, 5, 7, 9$, etc. The model suggests that growing polymer chains would terminate, in the case of Hp 2-1, when two α^1 units have combined with β units or, in the case of Hp 2-2, when the α^2 unit at the growing end of a polymer chain com-

bines with the initial β unit in the chain, thereby forming a circular protein. The polymerization of α and β units in the Hp 2-1 and Hp 2-2 phenotypes appears to confer additional antigenic determinants on these haptoglobin polymers which are not present on the Hp 1-1 molecule (Korngold, 1963). A structural basis for haptoglobin polymerization is discussed in greater detail elsewhere (Parker, 1963). Of interest is the general similarity between the proposed structure of haptoglobin and that postulated for specific antibodies (Edelman and Benacerraf, 1962).

Relative Quantitation of Haptoglobin Components

By means of densitometry after vertical starch gel electrophoresis (Fig. 2),

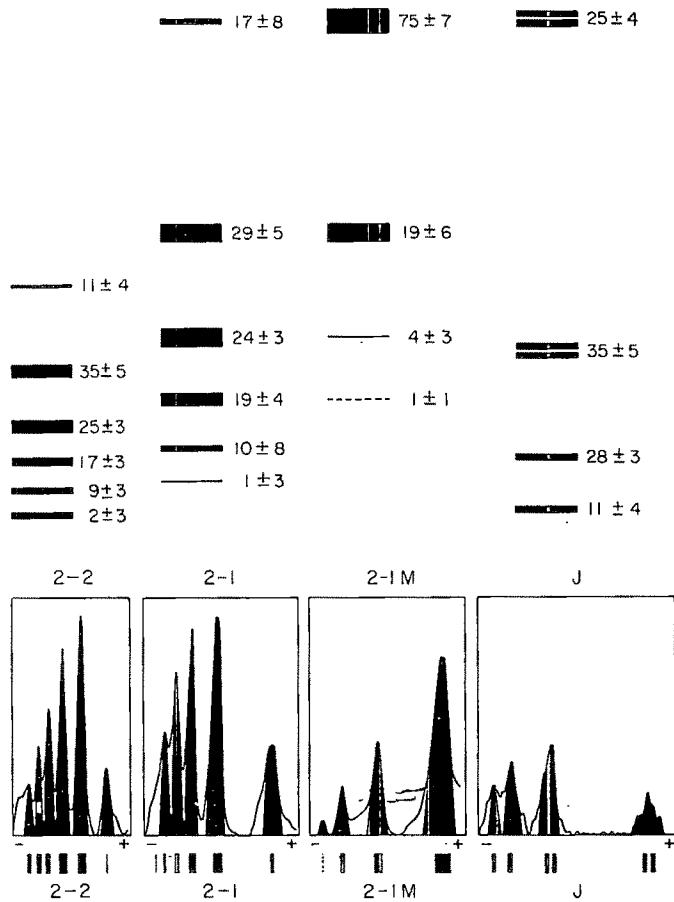


FIG. 2. Determination of relative concentration of individual haptoglobin components in the Hp 2-2, Hp 2-1, Hp 2-1M, and Hp J (Johnson) phenotypes. Densitometry after starch gel electrophoresis. Upper portion: calculated relative concentration and standard deviation. Lower portion: characteristic densitometric tracing for each phenotype. The starch gels were sliced into layers of 1.5 mm thickness for use in the densitometer. The Hp 1-1 pattern (cf. Fig. 1), in which all of the haptoglobin is present as a single component, is not shown.

it has been possible to determine the relative concentrations of the individual haptoglobin components. For this analysis 9 different sera phenotype Hp 2-2, 12 of Hp 2-1, 8 of Hp 2-1M, and 3 of Hp Johnson (Hp J) were used; 20, 23, 8, and 5 tracings, under various light intensities of the densitometer (Photovolt, Densicord), were obtained for each phenotype, respectively. The Hp Johnson phenotype is described in a subsequent section. An amount of hemoglobin sufficient to saturate the haptoglobin was added to each sample prior to electrophoresis. Since the benzidine reaction detects the peroxidase activity of the haptoglobin-hemoglobin complexes, it is only indirectly a measure of haptoglobin concentration. A basic assumption in these determinations is that the benzidine-positive components accurately reflect the haptoglobin concentration in the individual components of the starch gel patterns. This assumption will remain in doubt until the nature of the bond between haptoglobin and hemoglobin is established. However, it is of interest that the starch gel patterns of purified haptoglobins which have been stained directly for protein with amido black (Connell and Smithies, 1959) closely parallel the benzidine pattern.

The upper portion of the diagram (Fig. 2) illustrates the starch gel pattern observed for the four phenotypes. The calculated relative concentration and standard deviation for each component (total haptoglobin = 100) are given beside each band in the diagram. The characteristic tracing for each phenotype by densitometry is shown beneath the starch gel pattern. The multiple components in each phenotype are clearly resolved in the tracing; for comparison a diagram of the starch gel pattern is shown below each tracing. The outline on each tracing is the curve obtained directly from the recorder of the densitometer; the areas used in the calculation of the relative concentrations of each component are shaded. Because of slight trailing of benzidine-positive material in the haptoglobin region of the gel, the base line used in the area calculations for each phenotype was set as the lowest trough between peaks within the pattern. The values obtained for the standard deviation indicate that the tracings are reproducible and can be used to distinguish between the phenotypes. The relative concentration obtained in this manner was used to calculate the amounts of Hp 2 (α^2) units in the Hp 2-1 and Hp 2-1M phenotypes (table 1).

Activation Model for Haptoglobin Synthesis

Fig. 3 represents a possible genetic mechanism to account for the decreased production of the Hp 2 unit in the Hp 2-1M phenotype. According to this scheme the haptoglobin locus consists of a control gene *C* and a structural gene *Hp*. The control elements *C*¹ and *C*² in the genetic material produce substances *A*¹ and *A*² which act through the cytoplasm and which are necessary to activate the structural genes *Hp*¹ and *Hp*² for the production of haptoglobin. *A*¹ is assumed to have a higher affinity for *Hp*¹ than for *Hp*² and *A*² an equal affinity for both *Hp*² and *Hp*¹. (The assumption that *A*¹ has a higher affinity for *Hp*¹ than for *Hp*² is essential for the control hypothesis. No such assumption is necessary for *A*²; the hypothesis is also consistent if *A*² has a greater affinity for *Hp*² than for *Hp*¹.) In the genotype *C*¹*Hp*¹/*C*²*Hp*² (Fig. 3a), a sufficient amount of activators *A*¹ and *A*² is produced for normal synthesis of the Hp 1 and Hp 2 units, and the normal Hp 2-1 phenotype is observed in the starch gel. However,

TABLE 1. RELATIVE CONCENTRATIONS OF INDIVIDUAL HAPTOGLOBIN COMPONENTS

Band	Molecular structure in α^1 , α^2 and β chains	Hp 2-1		Hp 2-1M	
		Relative concentration in starch gel bands	Number α^2 units	Relative concentration in starch gel bands	Number α^2 units
1	$\alpha^1 \beta \alpha^1$	17	0	75	0
2	$\alpha^1(\beta\alpha^2)\beta\alpha^1$	29	29	19	19
3	$\alpha^1(\beta\alpha^2)_3\beta\alpha^1$	24	72	4	12
4	$\alpha^1(\beta\alpha^2)_3\beta\alpha^1$	19	95	1	5
5	$\alpha^1(\beta\alpha^2)_7\beta\alpha^1$	10	70		
6	$\alpha^1(\beta\alpha^2)_9\beta\alpha^1$	1	9		
Total			275		36

$$\frac{\alpha^2 \text{ units in Hp 2-1M}}{\alpha^2 \text{ units in Hp 2-1}} = \frac{36}{275} \approx \frac{1}{8}$$

Estimate by densitometry of the relative amounts of α^2 units (*i.e.*, the α polypeptide chains synthesized by the Hp^2 gene) present in the phenotypes Hp 2-1 and Hp 2-1M. The molecular structure of the bands in Fig. 1 is represented as composed of α^1 , α^2 , and β polypeptide chains in the sequence which most easily satisfies the observed electrophoretic pattern. If the haptoglobin bands are represented according to the theory of Allison (1959), a similar decrease in the amount of Hp 2 units in the Hp 2-1M phenotype is obtained.

TABLE 2. GENOTYPE AND CORRESPONDING PHENOTYPES BY STARCH GEL ELECTROPHORESIS FOR THE POSSIBLE COMBINATIONS OF CONTROL GENE (C) MUTATIONS

Genotype	Phenotype
$C^1 Hp^1/C^1 Hp^1$	1-1
$C^1 Hp^1/C^1-Hp^1$	1-1
C^1-Hp^1/C^1-Hp^1	0
$C^1 Hp^1/C^2 Hp^2$	2-1
$C^1-Hp^1/C^2 Hp^2$	2-1
$C^1 Hp^1/C^2-Hp^2$	2-1M
C^1-Hp^1/C^2-Hp^2	0
$C^2 Hp^2/C^2 Hp^2$	2-2
$C^2-Hp^2/C^2 Hp^2$	2-2
C^2-Hp^2/C^2-Hp^2	0

Note: C^1 , C^2 = active control gene
 C^{1-} , C^{2-} = inactive control gene

Recent evidence by Smithies, Connell and Dixon (1962a) indicates that the Hp^1 allele can be subdivided into two classes, Hp^{1F} and Hp^{1S} , although for the present purpose both may be considered as Hp^1 .

if a mutation occurs in C^2 so that it can no longer produce an active A^2 (genotype C^1Hp^1/C^2-Hp^2 , Fig. 3b), then a decreased amount of the Hp 2 unit will be produced and the Hp 2-1M pattern will result. A quantitative estimate

(table 1) indicates that approximately one-eighth of the normal amount of the Hp 2 gene product is present in the Hp 2-1M phenotype.

Conversely, the reciprocal heterozygote (C^1-Hp^1/C^2Hp^2 , Fig. 3c) will produce Hp 1 and Hp 2 units in approximately normal proportions, and therefore the observed phenotype will be indistinguishable from the normal Hp 2-1. Individuals homozygous for the control mutation ($C-Hp/C-Hp$, e.g., Fig. 3d) will produce no activators and therefore will synthesize no haptoglobin and will be of phenotype Hp 0. Table 2 lists the 10 possible genotypes and their corresponding phenotypes. The assumption of differential activity of the gene products of the control loci C^1 and C^2 is necessary in order to provide a biochemical mechanism for the appearance of the Hp 2-1M phenotype. In certain populations, such as Danish (Galatius-Jensen, 1960), Chinese (Steinberg *et al.*, 1961), Southeast Asia (Kirk and Lai, 1961), and Hutterite (Giblett, 1963), Hp 0 individuals have been observed in the absence of the Hp 2-1M phenotype. If, as seems likely, some of the cases of ahaptoglobinemia reported in these

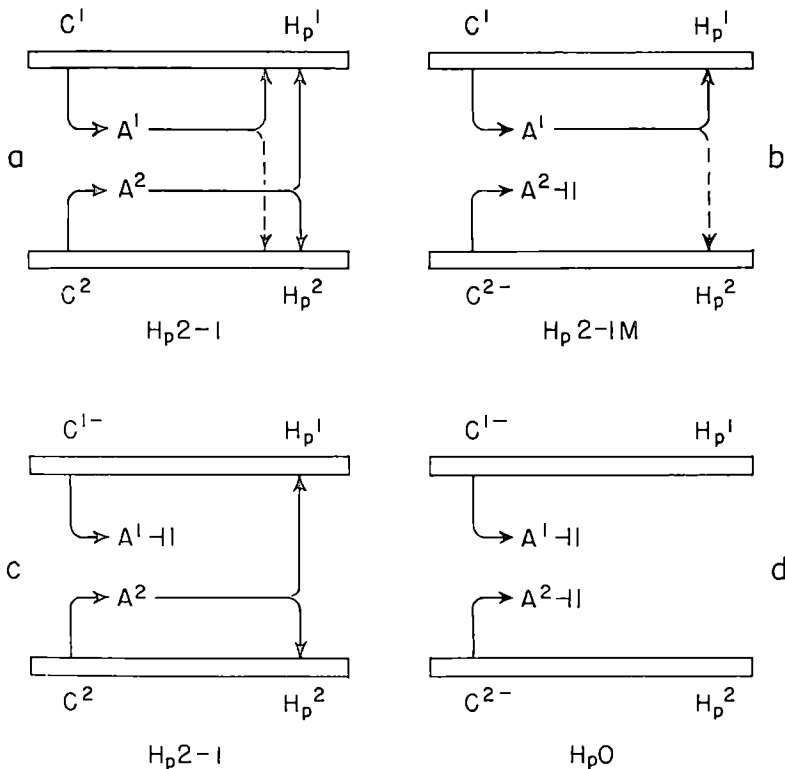


FIG. 3. Diagrammatic representation of hypothetical activation mechanism for the genetic control of haptoglobin production. The control gene C produces a substance A which activates the haptoglobin structural gene Hp . As shown, the corresponding phenotypic patterns observed by starch gel electrophoresis are:

- (a) Hp 2-1; genotype C^1Hp^1/C^2Hp^2
- (b) Hp 2-1M; genotype C^1Hp^1/C^2-Hp^2
- (c) Hp 2-1; genotype C^1-Hp^1/C^2Hp^2
- (d) Hp 0; genotype C^1-Hp^1/C^2-Hp^2

populations can be attributed to genetic causes, then it is necessary to postulate that the control loci may differ between Negro and non-Negro populations; in non-Negro populations, a common control locus C may be postulated for both of the structural genes Hp^1 and Hp^2 . In such populations, a C^-/C^- individual would have the phenotype Hp 0, whereas C^-/C^+ individuals would produce normal proportions of haptoglobin at the structural loci and would therefore show one of the three common phenotypes; in these populations, the control mutation is essentially equivalent to the recessive suppressor gene postulated by Sutton *et al.* (1959). Alternatively, the apparent absence of the Hp 2-1M phenotype in non-Negro populations can be explained if the C^- mutation is restricted to the C^1 locus in these populations.

By this analysis, based on absolute linkage of the control and structural gene loci for haptoglobin, it is possible to account for almost all of the reported data on the inheritance of the Hp 2-1M and Hp 0 phenotypes. The data of Giblett and Steinberg represent the segregation of the C^- mutation in 91 families of 488 individuals, of whom at least 110 carried the mutation. The three Hp 0 offspring discussed previously, whose phenotypes are inconsistent with the Hp^{2m} allele hypothesis, may be accounted for if the Hp 1-1 and Hp 2-2 parents are genotypically C^1Hp^1/C^1Hp^1 and C^2Hp^2/C^2Hp^2 , respectively. The only apparent exceptions to the control hypothesis are two children in the same pedigree (II-2 and II-6 in Giblett and Steinberg*, 1960) and the Hp 2-1M child reported by Barnicot *et al.* (1960), and these children must be considered illegitimate on the basis of the present form of the hypothesis.

Testing of the Hypothesis by Critical Matings

An important feature of the genetic aspect of the hypothesis is its susceptibility to test. A critical mating for the hypothesis is Hp 2-1M x Hp 2-1M, from which no children should be produced of Hp 2-1 phenotype. Giblett and Steinberg reported four such matings with 15 offspring (children II-2 and II-6 in Giblett and Steinberg (1960) were offspring of this mating type and have been excluded from the calculation). No Hp 2-1 children were found, and the observed ratio of Hp 1-1, Hp 2-1M and Hp 0 phenotypes (3:8:4) was not significantly different ($P > .50$) from the expected 1:2:1 ratio. A second critical mating for the hypothesis is Hp 2-1 x Hp 2-1, which should yield no Hp 2-1M offspring, although Hp 0 children are permissible if the Hp 2-1 parents are genotypically C^1Hp^1/C^2Hp^2 . In 13 such matings, Giblett and Steinberg found no Hp 2-1M and 4 Hp 0 offspring. The most critical mating is Hp 0 x Hp 0, from which all offspring should also show the Hp 0 phenotype; no instances of this mating type have been reported. Five matings involving haptoglobin-negative parents with haptoglobin-positive offspring have been observed by Barnicot *et al.* (1960) in a Nigerian population; however, it was the conclusion of these authors that the haptoglobin-negative phenotypes in this population resulted largely from environmental influences.

Four* additional mating combinations also provide tests of the control hy-

* On the particular facts of this pedigree, one or the other of II-2 and III-2 must be considered extramarital; the choice of II-2 as the illegitimate child was made arbitrarily. In addition, the classification of II-6 as Hp 2-2 is considered uncertain.

pothesis. Individual matings of types Hp 2-1 x Hp 2-2 and Hp 2-1 x Hp 2-1M should not produce offspring of both Hp 2-1M and Hp 0 phenotypes; similarly, individual matings of types Hp 2-1 x Hp 2-1 and Hp 2-1 x Hp 0 should not produce both Hp 1-1 and Hp 0 offspring. Drs. Giblett and Steinberg have kindly examined the matings in their family studies and have found three offspring who do not satisfy the hypothesis. In one case, a Hp 2-1 x Hp 2-1M mating produced one Hp 2-1 and two Hp 0 children; in the second case, a similar mating produced two Hp 2-1M and one Hp 0 children; in the third case, a Hp 2-1 x Hp 2-2 mating produced six Hp 2-2, one Hp 2-1M, and one Hp 0 children. The inconsistencies represented by these three matings and by the three offspring considered illegitimate cannot be reconciled by the present hypothesis; it will be shown that these inconsistencies can be resolved by assuming partial linkage or independent segregation of the control and structural gene loci. However, it is important to interpret these findings in relation both to the significantly higher number of Hp 0 children as compared to Hp 0 parents observed by Giblett and Steinberg and to the estimated illegitimacy rate of 8 per cent in American Negroes (Schacht and Gershowitz, 1961). In the pedigrees of Giblett and Steinberg, children found to be extramarital by any of 10 genetic markers or by the mother's admission were excluded from the study.

Testing of the Hypothesis by the Hardy-Weinberg Equilibrium

On the basis of the observed phenotypes among 183 parents of the 92 families studied by Giblett and Steinberg (one ahaptoglobinemic parent with Hb SC disease has been excluded), it is possible to calculate the expected distribution of phenotypes under conditions of Hardy-Weinberg equilibrium (table 3). In the calculation for case A, values for p and q were determined from the combined quantity $(p+q)$ by assuming that the frequency of the control mutation is the same at both the C^1 and C^2 loci ($p/q = r/s$). The observed phenotypes reveal a large excess of Hp 2-1M individuals, and application of the χ^2 test indicates a significant departure from Hardy-Weinberg equilibrium. If the assumption of equal frequencies for the C^1 and C^2 mutations is valid, then the cause of the lack of agreement with the equilibrium values is not clear. It is possible that the excess of Hp 2-1M phenotypes may represent a weighting of samples toward this phenotype; in addition, it has recently been suggested (Parker and Bearn, 1961) that U. S. Negro populations may not satisfy the conditions for genetic equilibrium at the Hp locus, since various selective factors in the North American environment appear to be operating against the characteristic high frequency of the Hp^1 allele in Negro populations.

If, however, the assumption is not made that the frequencies of the C^1 and C^2 mutations are equal, alternate values for p and q may be determined from $(p+q)$ and the number of Hp 2-1M individuals (table 3, case B). It is seen that the frequency q of the C^2-Hp^2 allele is considerably greater than that of the C^1-Hp^1 allele, in spite of the lower incidence of the Hp^2 structural gene in the population. It is also observed that the gene frequency values are now in reasonably good agreement with the distribution of phenotypes expected under Hardy-Weinberg equilibrium. This agreement suggests the possibility that the frequency of the control mutation may not be equivalent at the two loci.

TABLE 3. CALCULATION OF THE DISTRIBUTION OF HAPTOGLOBIN PHENOTYPES UNDER CONDITIONS OF HARDY-WEINBERG EQUILIBRIUM

Phenotype	Expected frequency	Expected No. Indiv.		Observed No. Indiv.
		A	B	
Hp 1-1	$r^2 + 2pr$	59.8	46.0	46
Hp 2-1	$2rs + 2ps$	72.9	63.0	73
Hp 2-2	$s^2 + 2qs$	31.1	41.0	31
Hp 2-1M	$2qr$	13.2	27.0	27
Hp 0	$p^2 + 2pq + q^2$	6.0	6.0	6
Total		183.0	183.0	183
$(p + q) = \sqrt{Hp\ 0} = 0.181$				
$s = 1 - \sqrt{Hp\ 1-1 + Hp\ 2-1M + Hp\ 0} = 0.343$				
$r = 1 - (p + q + s) = 0.476$				
Case A		Case B		
$p = 0.105$		$q = \frac{Hp\ 2-1M}{2r} = 0.155$		
$q = 0.076$		$p = 0.181 - q = 0.026$		
$\chi^2 = 17.6$		$\chi^2 = 4.0$		
$P < 0.001\ (DF = 1)$		$P = .05\ (DF = 1)$		
Note: p = frequency of C^1-Hp^1		r = frequency of $C^1\ Hp^1$		
q = frequency of C^2-Hp^2		s = frequency of $C^2\ Hp^2$		

Biochemical Comparison of the Structural Gene Products. Possible Origin of the Control Gene Mutation

An additional question of interest for the hypothesis is the comparison of the Hp 2 gene products represented in the Hp 2-1 and Hp 2-1M phenotypes. It does not yet appear possible to state with complete certainty whether these products are identical. Smithies (1960) and Smithies, Connell and Dixon (1962a) have reported a slightly reduced (1-2 per cent) electrophoretic mobility for Hp 2-1M as compared to Hp 2-1 after reductive cleavage. However, these authors also state that the treated Hp 2-1M phenotype is more easily distinguished from the Hp 2-1 type by the relatively reduced staining property of the band corresponding to the Hp 2-1M product in the urea-formate starch gel; it seems possible that the reduced staining property may reflect a decreased amount of the Hp 2 gene product in the Hp 2-1M phenotype, an interpretation which would be in agreement with the present hypothesis. In fact, the photograph and description of the urea-formate gel in Smithies *et al.* (1962a) are consistent with the suggestion that the Hp 2M product may be present in approximately one-eighth the amount of the normal Hp 2 product. It is also possible that the reduction in mobility may represent the presence in the Hp 2-1M individual studied by Smithies *et al.* (1962a) of a Hp 2 unit formed by the fusion of two Hp 1S units, rather than the Hp 1F-1S fusion generally observed (Smithies *et al.* (1962b)). The existence of such Hp 2 units has been postulated by these authors (1962b). It should be noted that a structural difference between the two Hp 2 gene products would not necessarily be incompatible with the hypothesis, since the genetic alteration might include both the control gene and the

structural gene. As previously discussed, Smithies *et al.* (1962b) have postulated the formation of the Hp^2 gene by unequal crossing-over at the Hp^1 locus. It is possible that the difficulty in mitotic pairing of homologous loci in individuals heterozygous for the smaller Hp^1 and larger Hp^2 genes could lead to a mutation in the region of these loci. In studies on naturally-occurring chromosome inversions in various species of *Drosophila*, Novitski (1946, 1961) has suggested that the increased chromosome breakage observed in inversion heterozygotes may result in part from a stress imposed by the paired configuration. In studies on a multiple-inversion chromosome in *Drosophila melanogaster*, Thompson (1962) has recently postulated that asynapsis interferes with normal mechanisms of chromosome repair and thereby results in the observed higher frequency of recognizable mutations. It is therefore possible that both the stress and the asynapsis of the looped configuration of the C^2Hp^2 locus which would result at each pairing of homologous chromosomes involving the C^1Hp^1 and C^2Hp^2 loci would favor the occurrence of a deletion at the C^2Hp^2 locus. On this basis, the alteration in the Hp 2-1M and Hp 0 phenotypes may be viewed as a possible deletion of the control locus C; depending upon the juxtaposition of the C and Hp loci, such a deletion could also affect the structural gene Hp^2 . The deletion mechanism might also provide a partial explanation for the high frequency of the C^2 mutation as determined by Hardy-Weinberg equilibrium; the observed relative frequencies (table 3, case B) of the C^1 and C^2 mutations suggest that the mutation is approximately six times more likely to occur at the locus C^2Hp^2 than at C^1Hp^1 .

Repression Model for Haptoglobin Synthesis

The present analysis has thus far been presented in terms of a control mechanism based on activation of the structural genes. If an attempt is made to apply a mechanism of repression, a model based on the theory of Jacob and Monod (1961) may be postulated. By this model, illustrated in Fig. 4, the haptoglobin operon Hp is assumed to include a regulator gene RG_a which synthesizes a repressor R_a for the operator gene O_b governing a repressor R_b for the Hp operon; the concept of the operator gene was introduced by Jacob *et al.* (1960) to describe a genetic element whose function is to initiate transcription of structural units by the adjacent (*cis*) structural gene locus. A prototype for such a model of interacting control elements has recently been presented (Fig. 5 in Monod and Jacob, 1961). If a mutation (the *i* mutation of Jacob and Monod, 1961) occurs in RG_a such that R_a is no longer active against O_b , then R_b will be synthesized and the Hp operon will be blocked. In diploid genotypes, the repressor product R_a^1 of the Hp^1 operon is assumed to have a higher affinity for O_b^1 than for O_b^2 . In the Hp 2-1M phenotype, a mutation is assumed to occur in RG_a^2 such that R_a^2 is inactive. R_a^1 will continue to repress both O_b^1 and O_b^2 although it will be less effective against O_b^2 , thereby causing the decreased synthesis of Hp 2 units characteristic of the Hp 2-1M phenotype. If the mutation in RG_a^2 is in fact a deletion, as has been tentatively discussed above, then such a deletion might also affect the structural gene Hp^2 and result in the production of a variant Hp 2 unit. By similar analyses the remaining three activation models of Fig. 2 can be translated into corresponding repression models

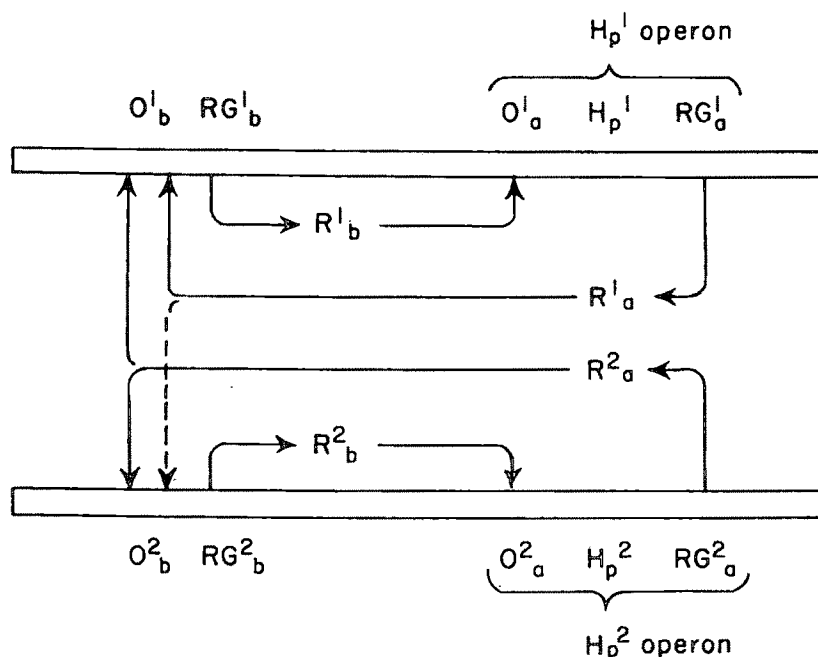


FIG. 4. Hypothetical model for the genetic control of haptoglobin production according to a repression mechanism. The regulator gene RG_b controls the activity of an operon containing the haptoglobin structural gene Hp and another regulator gene RG_a . The regulator gene RG_b belongs to a separate operon which is sensitive to the repressor R_a synthesized by RG_a . Trans action of the repressor R_b is omitted from the diagram.

for the determination of the respective phenotypes. The essential feature of the repression mechanism, which is formally equivalent to the activation model, is the postulated differential activity of repressors R^1_a and R^2_a . An analogous example of differential repressor activity is represented by the control system for arginine biosynthesis in *Esch. coli*, where noncoordinate repression of certain enzymes in the system has been observed (Vogel, 1961; Gorini, Gundersen, and Burger, 1961; Maas, 1961).

Possible Mutations in a Haptoglobin Operator Gene

The observation by Harris, Robson and Siniscalco (1958) of five Hp 2-2 offspring of Hp 1-1 x Hp 2-1 and Hp 1-1 x Hp 2-2 matings within a single pedigree raises the interesting possibility of a different type of control mutation, a mutation of the haptoglobin operator gene. In this pedigree, the three Hp 1-1 parents are a brother and two sisters; each of these parents has at least one Hp 2-2 child. The exceptional Hp 2-2 phenotypes may be explained if it is assumed that the Hp 1-1 parents are genotypically $C^1O^1Hp^1/C^2O^2Hp^2$, where O is the haptoglobin operator gene. O^2 represents an alteration (the o^0 mutation of Jacob and Monod, 1961) in the operator gene for the Hp^2 operon such that the operon is no longer active. In this situation, the Hp 2-2 offspring would be genotypically $C^2O^2Hp^2/C^2O^2Hp^2$. A similar result is obtained if the Hp 1-1 parental genotypes are $C^1O^1Hp^1/C^1O^1Hp^1$, since in either case the altered Hp

operon would be inactive. The deletion mechanism previously discussed suggests that the mutation would be more likely to occur at the O^2 locus. In practice, it would be extremely difficult to distinguish such an operator gene mutation from a deletion of the entire haptoglobin locus; in both instances, the result would of course be equivalent to the rare allele Hp^0 postulated by Harris *et al.* to explain the pedigree.

The interaction of the Hp 1 and Hp 2 structural gene products in heterozygous individuals suggests that haptoglobin operator gene mutations will be most easily detectable in these individuals; a large-scale population-screening project is presently under way for the collection of genetic and biochemical data to test various control hypotheses. It may be predicted, for example, that constitutive mutations of the haptoglobin operator gene (the o^c mutation of Jacob and Monod, 1961) will lead to the occurrence of inherited variations in the normal Hp 2-1 phenotype. Thus an o^c mutation in the Hp^1 operon, in which the Hp^1 operator gene is no longer capable of inhibition by the repressor, would be expected to result in the production of an increased amount of Hp 1 units relative to Hp 2 units; similarly, an o^c mutation in the Hp^2 operon would lead to an excess of Hp 2 units. Unfortunately, it is not possible to predict accurately the expected starch gel patterns for such mutations until a better understanding of the mechanism of haptoglobin polymerization has been achieved. It is possible that the o^c mutation in the Hp^1 operon would result in a phenotype similar to Hp 2-1M, since the proportion of Hp 1 to Hp 2 units would be approximately similar to the proportion in the Negro Hp 2-1M phenotype. However, the analysis of the Negro phenotype in terms of a regulator gene mutation (the i^- mutation of Jacob and Monod, 1961) is supported by the association of the Hp 2-1M and Hp 0 phenotypes in pedigree material, a finding which is inconsistent with an o^c mutation.

Correspondingly, an o^c mutation in the Hp^2 operon could yield, according to the kinetics of polymerization, either an unusually large number of Hp 2-1 polymer components or a mixture of Hp 2-1 and Hp 2-2 components. In the former case, it is likely that additional antigenic determinants (Korngold, 1963) would be present as a result of the increased concentration of slower moving Hp 2-1 polymers; it is possible to interpret the rare inherited haptoglobin phenotype described by Aly *et al.* (1962), which possesses such additional determinants, as an o^c mutation in the Hp^2 operator gene. In the latter case, the pattern would be similar to the phenotype reported by Harris, Robson and Siniscalco (1959), which contained both Hp 2-1 and Hp 2-2 components. Galatius-Jensen (1958) has also observed an anomalous phenotype, in which a band with the mobility of the fastest moving Hp 2-2 polymer component is present in an otherwise normal Hp 2-1 pattern. It is possible that these phenotypes represent instances of haptoglobin operator gene mutations; it seems unlikely, however, that Hp 2-2-type polymers would appear in the presence of free Hp 1-1 component. Alternatively, as described in the following section, a chromosome alteration may account for these phenotypes.

The Haptoglobin Phenotype Hp Johnson and Haptoglobin Evolution

The rare haptoglobin phenotype Hp J (Johnson) was originally described by

Giblett (1959b) and represents a modification of the Hp 2-1 phenotype which is different from that observed in Hp 2-1M, where only the relative intensities of the individual haptoglobin components are altered. The starch gel pattern of the Johnson phenotype (Giblett, 1961) shows not only an alteration in intensity, such that band 1 (*cf.* Fig. 1 and 2) is more prominent, but also a shift toward the cathode of the slower-moving components (bands 2-6 in the normal Hp 2-1 pattern, Fig. 1); in addition, bands 1 and 2 are each split into two components. In the present experiments it has been possible to examine serum from three individuals of phenotype Hp J, and similar alterations in intensity and mobility, as well as the splitting of bands 1 and 2, have been observed. The evidence of Smithies *et al.* (1962b) that the Hp^2 allele represents a partial gene duplication resulting from the fusion of two Hp^1 alleles by non-homologous crossing over has been discussed previously; these authors have also suggested that one of the polymerizing units in the Hp J phenotype may represent a triplication of the basic Hp 1 unit formed by crossing over after displaced synapsis between two Hp^2 alleles. However, consideration of the Johnson allele as a fusion of three Hp^1 genes raises certain difficulties. The starch gel pattern of the slower-moving Hp J components (bands 2-6) in the present experiments shows the same relative spacing observed in the normal Hp 2-1 phenotype; this result is not easily reconciled with the expected increase in size of the polymerizing unit which would occur by a fusion of three Hp^1 genes. The over-all decrease in mobility of the slow-moving Hp J components indicates, as has been suggested by Smithies (1960), that the modification involves an alteration in the charge of the Hp 2 unit. The observed starch gel pattern of the Hp J phenotype is consistent with a genotype containing three independent genes, each synthesizing a different haptoglobin unit. Thus, one gene may be considered to synthesize a normal Hp 1 unit, the second a Hp 1 unit (Hp^{1x}) of slightly altered charge, and the third a Hp 2 unit (Hp^{2x}) of altered charge. The splitting of band 1 in the starch gel pattern may then be interpreted as indicating the presence of the two Hp 1 units. The splitting of band 2 would represent the combinations of the altered Hp 2 unit with the normal Hp 1 unit and with the altered Hp 1 unit; the relatively large molecular weights of bands 3-6 in the polymer series of the Johnson phenotype would make the resolution of split components in these bands extremely difficult. The Johnson genotype could theoretically arise if one haptoglobin allele is a normal Hp^1 gene and the other a composite gene formed by crossing over between the Hp^1 and Hp^2 loci such that after crossing over the Hp^{1x} and Hp^{2x} genes lie on the same chromosome. The stress and asynapsis involved in the pairing of Hp^1 and Hp^2 loci at cell division have been discussed previously as a potential origin of the control mutation in the Hp 2-1M phenotype; it is also possible that such a mechanism may lead to the formation of the Johnson allele. The split components and the alterations in mobility in the starch gel pattern may be explained if the break points on the chromosomes are such that the final cross-over product excludes a small portion of both the normal Hp^1 and Hp^2 genes. This hypothesis is very similar to that already postulated by Smithies *et al.* (1962b) except that in the present case the triplication (*i.e.*, the presence of a Hp^1 and a Hp^2 unit on the same chromosome, which is formally equivalent to three Hp^1 units) does not lead to the synthesis of a single large

haptoglobin polypeptide chain, but rather to the production of discreet, slightly altered Hp 1 and Hp 2 units.

A similar hypothesis may also be relevant to the rare haptoglobin phenotype observed by Galatius-Jensen (1958) and by Harris *et al.* (1959); in this phenotype, however, the haptoglobin components correspond in mobility to those of the normal Hp 2-1 phenotype, except that the fastest moving polymer (band 2) is split into two components. It is possible that the genetic complement of such individuals may include a normal Hp^1 , a normal Hp^2 , and an altered Hp^2 gene.

The hypothetical Johnson allele is also of interest in considering the evolution of human proteins. The pattern of hemoglobin evolution discussed by Ingram (1961) and by Zuckerkandl and Pauling (1962) illustrates an effective route for protein evolution: the duplication of a particular gene permits one of the duplicated genes to continue to provide a protein essential to the organism, while the other gene locus is available for experimentation. In this way it may be considered that the gene for the γ -chain of hemoglobin was evolved by experimentation upon a duplicated α -chain gene; similarly, genes for the β - and δ -chains appear to have evolved by duplication of the γ and β loci, respectively. In the case of haptoglobin, however, it may be postulated that the mechanism for evolution has functioned less effectively; the attempt to duplicate the Hp^1 gene was imperfect and resulted in a fusion of two Hp^1 genes, so that a single protein unit (the Hp 2 unit) was produced, rather than two independent Hp 1 units. Therefore, the range of experimentation available to the haptoglobin gene was curtailed, since one of the duplicated loci was not available to insure the organism against radical alteration at the other locus. According to this hypothesis, the Johnson chromosome may be seen as a successful attempt of haptoglobin gene duplication, in which the two loci synthesizing independent units (Hp 1 \times and Hp 2 \times) are present on the same chromosome; in this situation evolutionary experiments at one of the loci are less likely to confront the organism with the absence of an effective haptoglobin unit.

PARTIAL LINKAGE OR INDEPENDENT SEGREGATION OF THE CONTROL AND STRUCTURAL GENE LOCI

In each of the proposed models for the inheritance of the haptoglobin phenotypes, it has been convenient to regard the complex of control gene and structural gene as forming a single allele. Therefore, as employed in the calculation of the Hardy-Weinberg equilibrium (table 3), the present system may be considered equivalent to four alleles at the haptoglobin locus; however, it is through the use of the concept of control genes and structural genes that a biochemical mechanism may be postulated for the observed phenotypes Hp 2-1M and Hp 0. If the control and structural genes are partially linked or segregate independently, then the present hypothesis must be elaborated to account for the observed phenotypes. Partial linkage would allow the formation of 36 possible genotypes. On the basis of the mode of action previously proposed for the gene products at each control locus, 34 of the 36 genotypes may be assigned unique phenotypes (table 4); in addition, it is probable that the genotype C^1Hp^1/C^1Hp^2 would have

TABLE 4. GENOTYPES AND CORRESPONDING PHENOTYPES BY STARCH GEL ELECTROPHORESIS FOR THE 36 POSSIBLE COMBINATIONS OF CONTROL GENE (*C*) AND STRUCTURAL GENE (*Hp*) LOCI, ASSUMING PARTIAL LINKAGE OF THESE LOCI. IF THE CONTROL AND STRUCTURAL LOCI SEGREGATE INDEPENDENTLY, THERE ARE 30 INDEPENDENT GENOTYPES, SINCE, E. G., C^1Hp^1/C^2Hp^2 IS EQUIVALENT TO C^2Hp^1/C^1Hp^2

Genotype	Phenotype	Genotype	Phenotype
C^1Hp^1/C^1Hp^1	1-1	C^1Hp^2/C^1Hp^2	0
C^1Hp^2	2-1M	C^2Hp^1	2-1
C^1Hp^1	1-1	C^2Hp^2	2-2
C^1Hp^2	2-1M	C^2Hp^1	0
C^2Hp^1	1-1	C^2Hp^2	0
C^2Hp^2	2-1	C^2Hp^1/C^2Hp^1	1-1
C^2Hp^1	1-1	C^2Hp^2	2-1
C^2Hp^2	2-1M	C^2Hp^1	1-1
C^1Hp^2/C^1Hp^2	2-2	C^2Hp^2	2-1
C^1Hp^1	2-1M	C^2Hp^2/C^2Hp^2	2-2
C^1Hp^2	2-2	C^2Hp^1	2-1
C^2Hp^1	2-1	C^2Hp^2	2-2
C^2Hp^2	2-2	C^2Hp^1/C^2Hp^1	0
C^2Hp^1	2-1M	C^2Hp^2	0
C^2Hp^2	2-2	C^2Hp^2/C^2Hp^2	0
C^1Hp^1/C^1Hp^1	0		
C^1Hp^2	0		
C^2Hp^1	1-1		
C^2Hp^2	2-1		
C^2Hp^1	0		
C^2Hp^2	0		

Note: C^1 , C^2 = active control gene

Note: C^1 -, C^2 - = inactive control gene

TABLE 5. SEGREGATION OF THE CONTROL AND STRUCTURAL GENE LOCI

Parents		Children	
Genotype	Phenotype	Genotype	Phenotype
A. C^1Hp^1/C^2Hp^2 x C^1Hp^1/C^2Hp^2	Hp 2-1 x Hp 2-1M	C^1Hp^2/C^1Hp^1 C^1Hp^1/C^2Hp^2	Hp 2-1M Hp 0
B. C^1Hp^1/C^2Hp^2 x C^1Hp^2/C^2Hp^2	Hp 2-1 x Hp 2-2	C^1Hp^1/C^1Hp^2 C^1Hp^1/C^2Hp^2	Hp 2-1M Hp 0
C. C^1Hp^1/C^1Hp^1 x C^1Hp^1/C^2Hp^2	Hp 1-1 x Hp 2-1	C^1Hp^1/C^1Hp^2	Hp 2-1M

Possible inheritance of haptoglobin phenotypes on the basis of partial linkage or independent segregation of the control and structural loci for haptoglobin synthesis. In each case, a genotype consistent with the observed phenotype has been assigned to each individual. In A and B, the observed offspring combinations are inconsistent with the phenotypes of the parents if the control and structural loci are regarded as forming a single allele; in C, the Hp 2-1M child must be considered illegitimate on the basis of the allele hypothesis.

the phenotype Hp 2-1M and that the genotype C^2Hp^1/C^2Hp^2 would have the phenotype Hp 2-1. The Hp 2-1M phenotype for the genotype C^1Hp^1/C^1Hp^2 indicates the possible occurrence of this phenotype without a control mutation; in this situation, it would not be possible to explain the absence of the Hp 2-1M phenotype in non-Negro populations by an extremely low frequency for the control mutation in these populations. If the genotype C^1Hp^1/C^1Hp^2 is expressed by the phenotype Hp 2-1, this restriction is avoided.

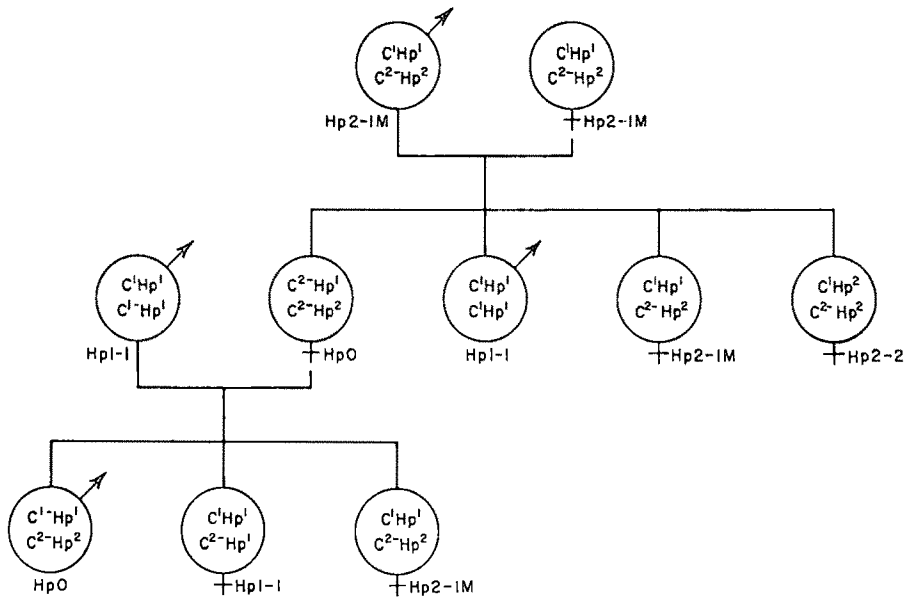


FIG. 5. Inheritance of haptoglobin phenotypes on the basis of partial linkage or independent segregation of the control and structural gene loci for haptoglobin synthesis. The members of this pedigree have been selected from a larger pedigree in Giblett and Steinberg (1960). Beneath each symbol is given the observed haptoglobin phenotype of the individual; within each symbol is given a possible genotype of the individual. It is seen that the observed phenotypes reveal no inconsistency with the assumed mode of inheritance. If, however, the control and structural loci are regarded as forming a single allele, the two inconsistencies described in the text for this pedigree are apparent. The genotypes of the parents in the first generation must remain as shown; therefore, the Hp 2-2 child and either the Hp 0 child in the second generation or the Hp 1-1 child in the following generation must be considered illegitimate. These children correspond to II-6, II-2, and III-2, respectively, in Giblett and Steinberg (1960).

If segregation of the control and structural gene loci is assumed, it becomes possible to resolve the inconsistencies in haptoglobin inheritance which arise when these loci are treated as forming a single allele. Thus, in the pedigree of Giblett and Steinberg, the two children who were necessarily illegitimate on the basis of the single allele hypothesis can be assigned compatible genotypes if segregation is permitted (Fig. 5); in a similar manner it is possible to resolve the two mating types which produced inconsistent offspring combinations (table 5, A and B), as well as the Hp 2-1M child observed by Barnicot *et al.* (table 5, C).

Allelism versus Segregation of the Control and Structural Gene Loci

Despite its greater latitude, certain experimental tests previously applied to the allele hypothesis can also be applied to the segregation hypothesis. For example, Hp 0 x Hp 0 matings should again produce only Hp 0 offspring; also, no children of Hp 2-1 phenotype should be produced by Hp 2-1M x Hp 2-1M matings (it is evident from table 4 that the Hp 2-1 phenotype must represent at least one C² gene, whereas a C² gene cannot be inherited from a Hp 2-1M

parent). Conversely, by similar reasoning from table 4, Hp 2-1 x Hp 2-1 matings should again produce no Hp 2-1M offspring. Therefore, both hypotheses satisfy these tests equally well and serve to explain most of the observed data. Matings which may be used to distinguish between the hypotheses of allelism and segregation are those already described, in which certain combinations of offspring are prohibited if the control and structural gene loci are regarded as a single allele. Additional distinctions are possible in three other mating types:

1. Hp 2-1M x Hp 2-1M, from which Hp 2-2 offspring are possible if the loci show segregation but are prohibited if the loci form a single allele (Fig. 4). In four such matings Giblett and Steinberg found one Hp 2-2 child among 17 offspring.
2. Hp 1-1 x Hp 2-1, from which Hp 2-1M children are possible by the segregation hypothesis but are prohibited by the allele hypothesis. In 15 such matings Giblett and Steinberg found no Hp 2-1M children among 48 offspring. However, the Hp 2-1M child of Barnicot *et al.* is an offspring of this mating type.
3. Hp 1-1 x Hp 2-1M, from which Hp 2-1 children are possible by the segregation hypothesis but are prohibited by the allele hypothesis. In six such matings Giblett and Steinberg observed no Hp 2-1 children among 15 offspring.

The data presently available appear insufficient to make a definite decision between allelism and segregation. The biochemical nature of the genetic material, the occurrence of the inconsistent Hp 2-2 and the Hp 2-1M children and the presence of the three matings producing combinations of offspring inconsistent with the allele hypothesis provisionally suggest partial linkage or independent segregation of the control and structural loci. The assumption of partial linkage, as opposed to independent segregation, is favored by the deletion mechanism previously discussed.

For the case of partial linkage or independent segregation of these loci, it is possible to compare the observed distribution of phenotypes with that expected under conditions of Hardy-Weinberg equilibrium (table 6). The expected distribution of genotypes is readily obtained from the binomial expansion of $[(p + q)(a + b + c + d)]^2$; the haptoglobin phenotype corresponding to each genotype is given in table 4. The values for p and q were determined from the number of Hp^1 and Hp^2 structural genes in the sample, excluding the six Hp 0 individuals, whose structural gene complement could not be determined. The value $(b + d)$ was calculated as shown; the symmetry of the binomial distribution in this case did not permit the calculation of individual values for b and d , and the combined value $(b + d)$ was employed throughout the calculation. The value for a was determined as the root of the quadratic equation involving p , $(b + d)$, and the observed frequency of the Hp 2-1M phenotype. By this method of calculation, the observed distribution of phenotypes is in good agreement with that expected under Hardy-Weinberg equilibrium. If, conversely, the quadratic equation involving p , q , and the frequency of the Hp 2-1 phenotype is solved for c , alternate values for c and a [$a = 1 - (c + b + d)$] are obtained which result in an expected equilibrium distribution in poor agreement ($P > .001$) with the observed distribution.

TABLE 6. CALCULATION OF HARDY-WEINBERG EQUILIBRIUM DISTRIBUTION OF HAPTOGLOBIN PHENOTYPES ON THE BASIS OF SEGREGATION OF THE CONTROL AND STRUCTURAL LOCI

Phenotypes	Expected Frequency	Expected No. Indiv.	Observed No. Indiv.
Hp 1-1	$p^2 - p^2(b + d)^2$	52.0	46
Hp 2-1	$2cpq (2 - c)$	60.6	73
Hp 2-2	$q^2 - q^2(b + d)^2$	37.2	31
Hp 2-1M	$2apq (a + 2b + 2d)$	27.1	27
Hp 0	$(b + d)^2$	6.0	6
		182.9	183
$p = 0.542$	$a = 0.395$		$\chi^2 = 4.3$
$q = 0.458$	$c = 1 - (a + b + d) = 0.424$		$P > .10 (DF = 2)$
	$(b + d) = \sqrt{Hp\ 0} = 0.181$		

Note: p = frequency of Hp^1

q = frequency of Hp^2

a = frequency of C^1

b = frequency of C^{1-}

c = frequency of C^2

d = frequency of C^{2-}

$$p + q = 1$$

$$a + b + c + d = 1$$

A similar principle, the genetically-determined control of haptoglobin production, is involved in each of the models which have been discussed. It is not known whether the control gene operates by activation or by repression. Evidence from bacterial and viral genetics indicates that the mechanism of control at this level is one of repression; the unlikelihood of obtaining genetic evidence on this point from human data suggests that the proper alternative must be chosen on the basis of the chemical isolation of the appropriate gene products.

CONCLUSION

The hypothesis of Giblett and Steinberg suggests that the inheritance of the serum haptoglobin phenotypes Hp 2-1M and Hp 0 is determined by a third allele Hp^{2m} at the haptoglobin locus. In the present report a possible alternate approach to haptoglobin inheritance has been developed involving elements of both structure and control in the genetic material. By this hypothesis the haptoglobin locus is considered to consist of a control gene C and a structural gene Hp ; by a mechanism of activation or repression, the control gene regulates the activity of the structural gene and thus determines the amount of haptoglobin synthesized. The observed pattern of the phenotype Hp 2-1M appears to represent a reduced synthesis of the Hp 2 gene product; the phenotype Hp 0 represents the essentially complete absence of haptoglobin synthesis. It is therefore suggested that these genetically-determined variations in haptoglobin production reflect a mutation in the control element C , such that the altered control locus C^- can no longer activate haptoglobin synthesis. The C^- mutation is equivalent to the i^- regulator gene mutation described in bacterial genetics (Jacob and Monod, 1961). On this basis, it is possible to assign a unique phenotype to

each of the possible haptoglobin genotypes. Individuals homozygous for the control mutation will produce no haptoglobin. Individuals heterozygous for the control mutation but homozygous for the structural genes Hp^1 or Hp^2 will produce haptoglobin of the common phenotypes Hp 1-1 or Hp 2-2, respectively, whereas individuals heterozygous for both the control mutation and the structural genes will be of phenotype Hp 2-1 (genotype C^1Hp^1/C^2Hp^2) or Hp 2-1M (genotype C^1Hp^1/C^2Hp^2). The occurrence of the Hp 2-1M phenotype can be explained by assuming a differential activity of the control loci C^1 and C^2 ; in populations where the Hp 0 phenotype is observed in the absence of the Hp 2-1M phenotype, this assumption is unnecessary. The possible formation of the Hp^2 gene by unequal crossing-over at the Hp^1 locus suggests that the control mutation may involve a deletion of the regulator gene locus; such a mutation may affect both the regulator and structural genes. Additional rare haptoglobin phenotypes such as Hp Johnson are also discussed, and the possibility of a haptoglobin operator gene mutation is considered.

The available data on the inheritance of the haptoglobin phenotypes have been examined from two points of view; in the first case, the control and structural genes are regarded as forming a single allele; in the second case, the possibility of partial linkage or independent segregation of these genes is considered. Both hypotheses are susceptible to genetic analysis. Gene frequency values were determined for each hypothesis, and the observed distribution of phenotypes was found to be in reasonable agreement with that expected under Hardy-Weinberg equilibrium. The calculated frequencies for the single allele hypothesis suggest that the control mutation is more likely to occur at C^2 than at C^1 . By the single allele hypothesis, there are three children who must be considered illegitimate; there are also three matings which have produced combinations of offspring inconsistent with the hypothesis. Although these inconsistencies can be resolved by the hypotheses of partial linkage or independent segregation, it seems improbable that either form of the control hypothesis as presented in this paper is correct in all its details. The final clarification of the complex problem of haptoglobin inheritance must await the accumulation of additional genetic and biochemical data.

SUMMARY

Application of the concepts of genetic regulatory mechanisms developed in bacteria allows the rare phenotypes Hp 2-1M and Hp 0 in the human serum haptoglobin system to be analyzed in terms of a mutation in a regulator gene at the haptoglobin locus. The hypothesis can be tested by certain critical matings. The limited genetic and biochemical data presently available are compatible with the hypothesis. In addition, the possibility of a haptoglobin operator gene mutation is discussed.

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The Blood Groups of a Further Family with Nail-Patella Syndrome

G. B. ELLIOTT, MARION LEWIS, HIROKO KAITA
AND BRUCE CHOWN

Calgary Medical Centre,
Calgary, and Rh Laboratory,
Winnipeg, Canada

IN 1955 Renwick and Lawler recognized linkage between the ABO and nail-patella loci. In their most recent paper on the subject (Lawler, Renwick, Hauge, Mosbech and Wildervanck, 1958) they estimated the recombination value to be 9.6 ± 2.4 per cent with no detectable heterogeneity between families with respect to this value. In the family set out in Fig. 1 the blood group data are compatible with linkage between the two loci and with the recombination value of Lawler *et al.*, the crude linkage-recombination ratio for the two loci being 5:1.

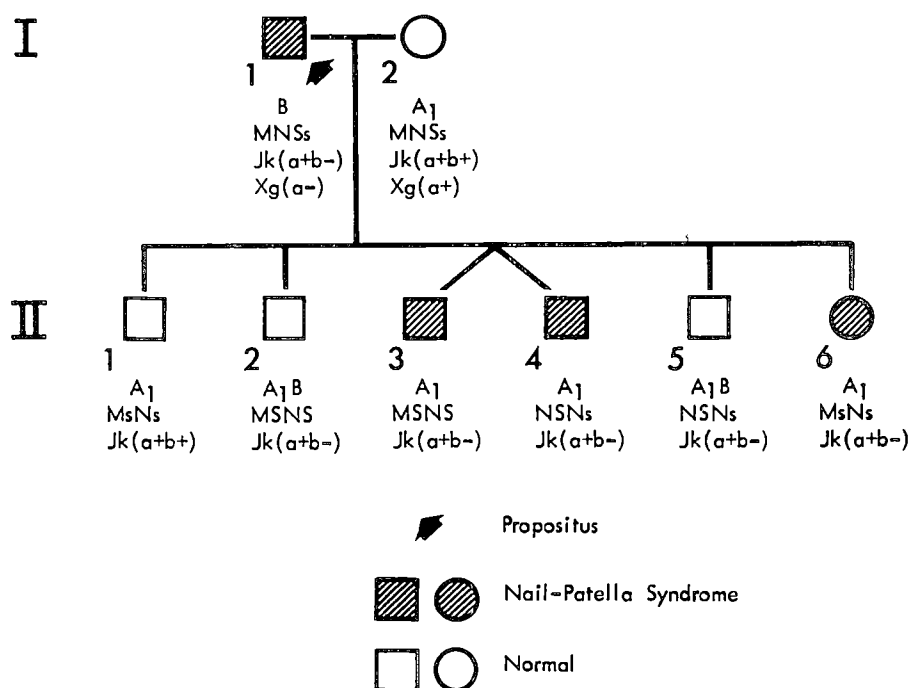


FIG. 1. Pedigree of family with nail-patella syndrome showing segregating blood groups. All members of generation II are Xg(a+). All members of both generations are: P+; C—, C^w—, c+, D—, E—, e+, f+; K—, k+, Kp(a—b+); Fy(a—b+); Lu(a—b+); Bu(a—).

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A detailed clinical description of this family has been published (Elliott, Elliott and Kindrachuk, 1962). The family is English; no other members live in North America.

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Hereditary Factors in Adiposis Dolorosa (Dercum's Disease)

HENRY T. LYNCH¹ AND WILLIAM L. HARLAN

*Department of Neurology,
Evansville State Hospital,
Evansville, Indiana*

ADIPOSIS DOLOROSA (Dercum's disease) is a condition wherein the chief clinical manifestations include obesity and painful subcutaneous lipomata. This combination was first described by Dercum (1892) in three patients. It is of interest that the patients were all females and a detailed family history in each case was negative.

A review of the literature to date is noteworthy for a paucity of patients with a positive family history. The only familial report we have been able to obtain is that of Keusch (1936) who recorded the disease in two sibs. Gates (1946) was impressed with the frequency of occurrence in females and expressed the thought that further elucidation in the manner of inheritance was required. Steiger, Litvin, Sasche and Durant (1952) described adiposis dolorosa in post-menopausal females with onset between the ages of 35 and 50 years. The possibility of a specific endocrine factor of etiologic importance was considered, but an endocrine survey was inconclusive. Winkelman and Echel (1925) suggested that a "pluriglandular" involvement is present. They based this suggestion upon a detailed necropsy study of their own and a review of 15 additional necropsy reports from the literature. They further noted a high frequency of interstitial neuritis in the nerve filaments of the fat nodules and speculated that this finding might well provide the basis for the sensation of pain in this condition. It is of interest that 12 of the 15 postmortem cases reviewed were females and their own case was also a female. They stated that the condition occurs five times as frequently in females as in males. The only therapy thus far of value has been surgical excision of the most symptomatic lipomata and weight reduction when indicated.

MATERIAL AND METHODS

The purpose of this investigation was to study the mode of inheritance of adiposis dolorosa and to gain further knowledge of the range of phenotypic expression of the gene through medical appraisal of all surviving members of two families where this disease is manifest. The two families were extensively investigated after confirmation of the diagnosis of Dercum's disease in the respective propositi. Diagnosis was based upon a careful history of the onset and development of painful subcutaneous nodules and a detailed physical examina-

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¹Present address: 4101 Bartman Drive, Omaha, Nebraska.

tion. Tissue was then obtained for pathologic examination. All available relatives were subsequently examined and records of pathologic examination of subcutaneous nodules were obtained if available.

RESULTS

Family A: The proband (Fig. 1, III-2) is a 26 year old white man who

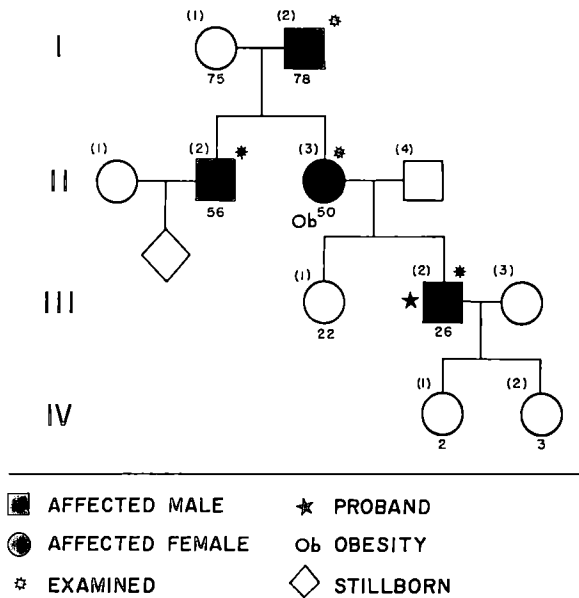


FIG. 1. Pedigree of family 1.

presented with a complaint of severely painful nodules, numbness, and weakness of the upper extremities. The onset of these complaints was at age 20 and signs and symptoms have been progressive since that time. Some 150 lipomata had been surgically excised during three hospitalizations. He still had many subcutaneous nodules over the entire body with the exception of the head. The nodules were more prominently distributed over the extremities and gluteal region and measured approximately 2 x 2 cm. in size. He had difficulty in assuming a position of comfort since pain was readily evoked by light palpation over the nodules. The patient was not obese. There was sensory loss over the ulnar nerve distribution bilaterally, secondary to lipomata impinging upon this nerve in the olecranon region. There was no significant motor deficit. The remainder of the physical examination was within normal limits. Pathologic specimens of the subcutaneous nodules were interpreted as being benign lipomata. Fig. 2 is a general view of the proband, and Fig. 3 shows the nodules in greater relief.

Positive diagnoses of adiposis dolorosa were also made on the proband's mother (Fig. 1, II-3), who was quite obese, his maternal uncle (II-2), and his maternal grandfather (I-2). Fig. 4 shows the maternal grandfather and careful inspection reveals the presence of nodules, particularly on the volar surfaces of the arms and on the anterior surface of the abdomen. The mother, uncle and

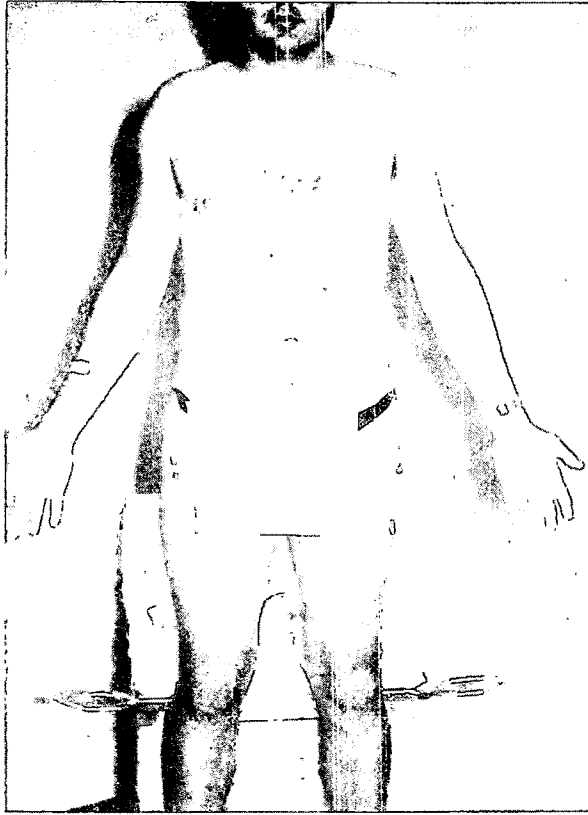


FIG. 2. View of proband, family 1. Note absence of obesity and mapping on right arm illustrating sensory impairment over the ulnar nerve distribution.

grandfather had fewer subcutaneous nodules than the proband, and symptoms of pain were of lesser magnitude, but definitely present.

Family B: The proband (Fig. 5, II-3) is a 49 year old white woman who is markedly obese. Painful subcutaneous nodules first appeared at age 45 and pain has since been progressive. She had a cholecystectomy in 1958 because of cholelithiasis. On physical examination there was marked obesity and multiple large painful subcutaneous nodules, measuring approximately 4 x 4 cm., were distributed over the entire body except for the head. On histologic examination one of the lesions showed the characteristics of a benign lipoma.

The proband's mother (Fig. 5, I-1) was markedly obese and had painful subcutaneous lipomata. The proband's two brothers (II-1 and II-2) also have subcutaneous lipomata, but these are not painful to palpation. Their physical examinations were otherwise within normal limits and they were not obese.

DISCUSSION

This is believed to be the first investigation wherein sufficient information has been obtained to justify postulation of the mode of inheritance of adiposis dolorosa, at least in two families.

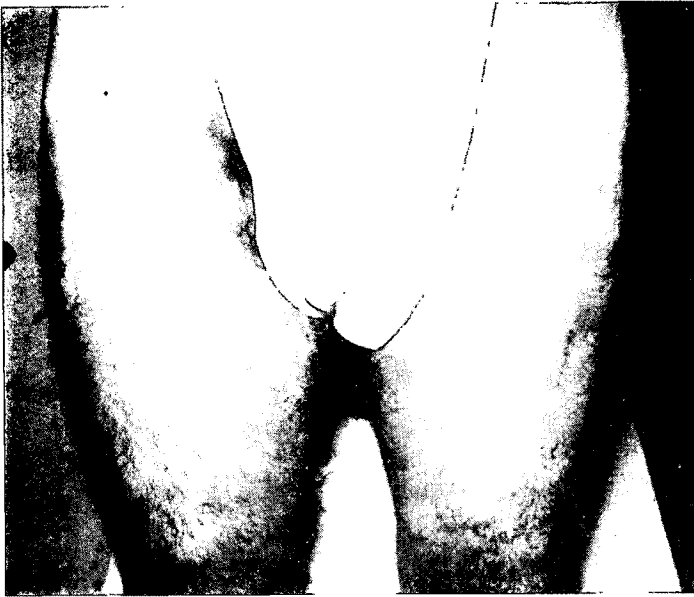


FIG. 3. Close up of subcutaneous lipomata over the anterior thighs.

Examination of the pedigree of Family A (Fig. 1) shows that Dercum's disease has been transmitted from parent to child through three generations. In Family B an affected mother transmitted the condition to all three of her children. Both males and females are affected, and sex-linkage is ruled out by the transmission of the disease from father to son in Family A. These findings suggest that adiposis dolorosa in these families is caused by an autosomal dominant gene.

The historical and physical findings suggest that there is variable expressivity of the postulated gene. The only individual with obesity in Family A is the mother of the proband. Symptoms of pain are considerably less manifest in the mother, the uncle and the grandfather than in the proband. In Family B both affected females are markedly obese and present the full clinical manifestations of the disease, while the males show only subcutaneous lipomata without the addition of pain. The range of clinical variation and the pattern of transmission therefore suggest the action of a dominant gene with variable expressivity.

In view of the marked obesity of the female members of both families, a sex-influenced factor cannot be excluded. This might well be an endocrine factor operative only in females which enhances the expression of the gene and produces obesity. This is certainly suggested by the marked obesity of affected females and the normal habitus in affected males, but our data are not extensive enough to exclude the effect of chance. However, if we couple these findings with those in the literature, we note a frequency of the disease in females 5 to 6 fold greater than that reported in males. Virtually all of the reported females have been markedly obese. Our findings show that the expression of the disease is frequently mild in males, and many such individuals would not be recognized unless very careful physical examinations were conducted. Hence, we postulate



FIG. 4. Maternal grandfather of the proband, family 1. Note presence of subcutaneous lipomata over arms and torso.

a factor potentially critical for sex to account for the apparant sex disparity in this condition. Unfortunately, there have not been enough family studies to date to permit evaluation of this hypothesis.

The broad spectrum of manifestations of adiposis dolorosa as seen in these families, ranging from extreme obesity with intensely painful nodules to completely asymptomatic subcutaneous lipomata without obesity, emphasizes the need for detailed evaluation of every member of the family. Had the brothers of the proband in Family B been examined without benefit of knowledge of the diagnosis of adiposis dolorosa in their sister and mother, they almost certainly would have been diagnosed as manifesting familial lipomatosis, when in fact they represent a *forme fruste* of Dercum's disease. In the light of this, it might be wise to re-evaluate families in which familial lipomatosis has been diagnosed for the possible presence of individuals with manifestations of Dercum's disease. Similar ranges in phenotypic variation have been found in a number of diseases (*e.g.*, McNutt, Klingman, Lynch and Harlan, 1960). Such studies have shown that only through meticulous examination of each individual in the family can we assess the true mode of inheritance and comprehend the many subtle features of the condition which might otherwise be overlooked. The reasons for such

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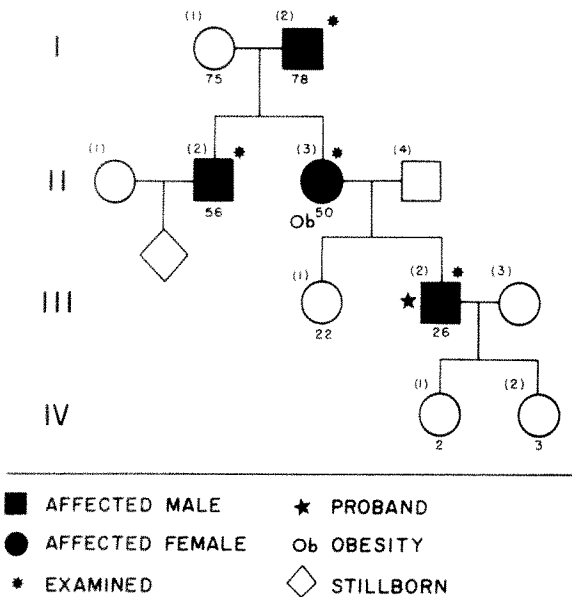


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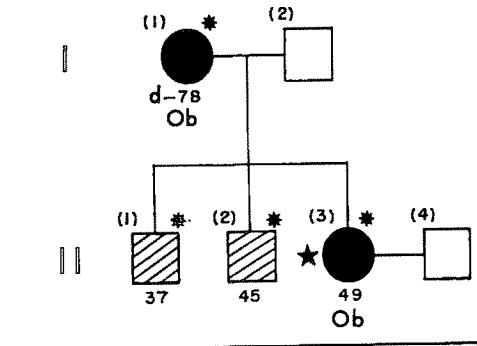
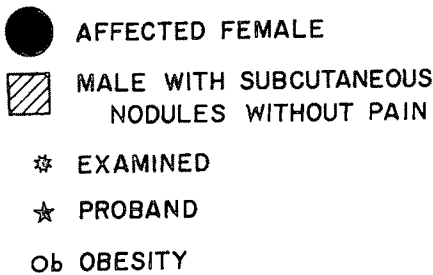


FIG. 5. Pedigree of family 2.



phenotypic variations of hereditary diseases are not completely clear. Such variation may be due, in part, to the action of the gene in differing biochemical milieu, to the presence of so-called modifying genes, or to environmental differences.

It seems clear that in the two families here described adiposis dolorosa may be ascribed to the action of a dominant gene with variable expressivity. However, most of the reports in the literature describe only a single case, and usually do not mention examination of relatives. It is entirely possible that affected relatives with minor clinical forms of adiposis dolorosa may have been overlooked in some previous studies because of lack of systematic examination of relatives. It is also possible that some apparently sporadic cases may represent new mutations to the postulated dominant gene. However, our experience with this disease is limited and we cannot exclude the possibility that an unknown proportion of sporadic cases may represent a different etiology.

SUMMARY

Two families with adiposis dolorosa (Dercum's disease) have been studied. In one family there are four cases in three generations, and in the second family there are four cases in two generations. The findings are interpreted as indicating the action of a dominant gene with variable expressivity. An hypothesis is advanced concerning the possible role of a sex-influenced factor.

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On the Estimation of the Frequency of Nonpaternity

JEAN W. MACCLUER AND WILLIAM J. SCHULL

*Department of Human Genetics,
University of Michigan Medical School,
Ann Arbor, Michigan*

SEGREGATION analyses, as well as current methods for the detection of linkage, tacitly assume that all children attributed to a given man and woman, and not clearly at variance genetically with them, are, in fact, their offspring. It can be readily shown, however, that there can exist children who although not the offspring of the man and woman in question are not demonstrably the children of others. The effect of this cryptic nonpaternity or nonmaternity on segregation analyses or linkage studies is difficult to evaluate, but must be related, in some manner, to the over-all frequencies of these events. It is of some importance then to be able to estimate these latter parameters. For a variety of reasons, but primarily because of its presumed greater occurrence, our attention will be directed solely to the estimation of nonpaternity. As we shall use this term, it is *not* synonymous with illegitimacy but rather it encompasses all instances in which the mother of a child incorrectly identifies the child's father. Thus, a child born out of wedlock but as a precondition for marriage would not be included among cases of nonpaternity provided that the mother correctly identified the father of the child.

A relationship exists between the over-all frequency of nonpaternity (λ) and the frequency (D) which can be detected on the basis of examinations of mother, child, and putative father with respect to a particular trait (or group of traits). Under certain conditions, namely, that the biological or "true" father be chosen at random from the population, and that the mother and putative father be unrelated, the ratio, D/λ , can be shown to be a function of gene frequencies alone. With the upsurge in interest in small, primitive communities, many of which have unusual patterns of procreation, it is readily conceivable that two or more of these individuals (mother, "true" father, and putative father) may be related. A more general expression for D/λ , applicable to these situations, is desirable. It is the purpose of this report to derive this more general expression, and to present a method for the estimation of the over-all frequency of nonpaternity. The method also affords estimates of the frequencies of the genes associated with the trait which provides the basis for the paternal exclusion.

THE PROPORTION OF NONPATERNITY WHICH IS DETECTABLE

Intuitively, the proportion of nonpaternity which is detectable must vary with the mode of inheritance of the trait used to demonstrate nonpaternity. Most

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TABLE 1. A SUMMARIZATION OF THE DERIVATION OF A GENERAL EXPRESSION FOR D/λ

Putative father Genotype σ_p (1)	"True" father σ_t (2)	$P(\sigma_t \sigma_p)$ (3)	Mother φ (4)	$P(\varphi \sigma_t)$ (5)	(6)	Proportion of nonpaternity detectable (7)	Probability of detecting nonpaternity = $\Sigma (2) \times (4) \times (6) \times (7)$ (8)
AA	p^2	AB	$C_T \cdot q + C_o \cdot 2pq$	AA	$(\frac{1}{2})d_{TP} + d_o \cdot p^2$	$\frac{1}{2}$	$\frac{p^2}{2}(C_T \cdot q + C_o \cdot 2pq) (1 - (\frac{1}{2})d_T - (\frac{1}{4})d_T - d_o \cdot pq)$
				AB	$d_T + (\frac{1}{2})d_T + d_o \cdot 2pq$	$\frac{1}{4}$	
				BB	$(\frac{1}{2})d_{Tq} + d_o \cdot q^2$	$\frac{1}{2}$	
		BB	$C_o \cdot q^2$	AA	$d_o \cdot p^2$	1	$p^2(C_o \cdot q^2) (1 - (\frac{1}{2})d_T \cdot p - d_o \cdot pq)$
				AB	$d_T \cdot p + d_o \cdot 2pq$	$\frac{1}{2}$	
				BB	$d_T + d_T \cdot q + d_o \cdot q^2$	1	
BB	q^2	AA	$C_o \cdot p^2$	AA	$d_T + d_T \cdot p + d_o \cdot p^2$	1	$q^2(C_o \cdot p^2) (1 - (\frac{1}{2})d_T \cdot q - d_o \cdot pq)$
				AB	$d_T \cdot q + d_o \cdot 2pq$	$\frac{1}{2}$	
				BB	$d_o \cdot q^2$	1	
		AB	$C_T \cdot p + C_o \cdot 2pq$	AA	$(\frac{1}{2})d_{Tp} + p + d_o \cdot p^2$	$\frac{1}{2}$	$\frac{q^2}{2}(C_T p + C_o \cdot 2pq) (1 - (\frac{1}{2})d_T - (\frac{1}{4})d_T - d_o \cdot p \cdot q)$
				AB	$d_T + (\frac{1}{2})d_T + d_o \cdot 2pq$	$\frac{1}{4}$	
				BB	$(\frac{1}{2})d_{Tq} + q + d_o \cdot q^2$	$\frac{1}{2}$	

frequently this trait will be some serotype. Accordingly, we shall be primarily concerned with two genetically different situations. First, we will define the proportion of nonpaternity detectable on the basis of a trait presumed to arise as the consequence of a single pair of autosomal alleles *without* dominance. Second, we will define this proportion when the trait is presumed to arise as a result of a single pair of autosomal alleles *with* dominance. In both instances, we shall assume that the population in question satisfies the Hardy-Weinberg equilibrium conditions, and that the probability, λ , that the "true" father is incorrectly identified is a fixed value independent of genotype or person. Clearly, neither of these assumptions is apt to be precisely fulfilled in any real situation; however, of the two, the constancy of λ would appear to be the more tenuous.

Alleles Without Dominance

Consider, now, the case of two autosomal alleles without dominance, where three genotypes, AA, AB, and BB are distinguishable. Suppose that p is the frequency of the gene A and q ($= 1 - p$) is the frequency of the gene B. Under this system, or for that matter those systems to be considered subsequently, the genetic correlation, ρ , between the putative father and the "true" father can be expressed in terms of three probabilities, c_I , c_T , and c_0 , where (see Li and Sacks, 1954)

c_I = probability that the putative and "true" fathers have, at a given locus, two genes identical by descent;

c_T = probability that the putative and "true" fathers have one gene identical by descent;

c_0 = probability that the putative and "true" fathers have no genes identical by descent;

and $c_I + c_T + c_0 = 1$. The corresponding probabilities for the mother and the "true" father are d_I , d_T , and d_0 . The specific values to be assigned to these probabilities depend upon the biologic relationship which is postulated; these values are of themselves of no particular moment to our argument.

The derivation of a general expression for D/λ is summarized in table 1. It should be noted that nonpaternity can not be detected if (1) the "true" father is of the same genotype as the putative father, or (2) the putative father is of genotype AB. These cases have, therefore, been omitted from the table. It should also be noted that we have assumed that the "putative" father and mother are unrelated. This is a matter of convenience, and the restriction can be easily removed. Be this as it may, the probability of detecting a case of nonpaternity, D/λ , is equal to the sum of the four elements in column (8). After some manipulation, this expression reduces to

$$\frac{D}{\lambda} = pq [1 - (\frac{1}{2})c_T - c_I] [1 - (\frac{1}{2})d_I - (\frac{1}{4})d_T - d_0pq] + p^2q^2c_0d_I \quad (1)$$

where $[(\frac{1}{2})c_T + c_I]$ is the genetic correlation, ρ , between the putative father and the "true" father, and $[d_I + (\frac{1}{2})d_T]$ is the comparable figure^o for the mother and "true" father.

Of particular interest are several special situations which can be readily deduced from equation (1). These are the following:

Case 1. If the mother, "true" father, and putative father are all chosen at random, they will have no genes identical by descent. Thus,

$$c_I = d_I = 0; c_T = d_T = 0; c_O = d_O = 1; \text{ and}$$

$$\frac{D}{\lambda} = pq(1 - pq). \quad (2)$$

This expression has been previously given by Wiener (1931), and by Cotterman (1951).

Case 2. If the mother and "true" father are unrelated, but the "true" father is related to the putative father, then

$$d_I = d_T = 0; d_O = 1; \text{ and}$$

$$\frac{D}{\lambda} = pq[1 - (\frac{1}{2})c_T - c_I] (1 - pq)$$

$$\text{or } \frac{D}{\lambda} = pq(1 - \rho) (1 - pq), \quad (3)$$

where ρ is the correlation between putative and "true" fathers.

Case 3. If the putative and "true" fathers are unrelated, but the "true" father is related to the mother, then

$$c_I = c_T = 0; c_O = 1; \text{ and}$$

$$\frac{D}{\lambda} = pq[1 - (\frac{1}{2})d_I - (\frac{1}{4})d_T - d_Opq] + p^2q^2d_I. \quad (4)$$

Case 4. Finally, in a population which is completely inbred, all individuals have two genes identical by descent. Therefore

$$c_T = c_O = d_T = d_O = 0; c_I = d_I = 1; \text{ and}$$

$$\frac{D}{\lambda} = 0.$$

It is moot, of course, whether in this context nonpaternity has any biologic significance.

Alleles with Dominance

We turn now to the case of two alleles with dominance. There are only two recognizable phenotypes, and nonpaternity can be detected only when both the putative father and the mother are of the recessive phenotype, say *aa*. The general expression, arrived at in a manner analogous to the case of no dominance, is

$$\frac{D}{\lambda} = pq^3[(\frac{1}{4}) c_T d_T + q(c_O d_O + (\frac{1}{2}) c_T d_O + (\frac{1}{2}) c_O d_T)], \quad (5)$$

where p is the frequency of the gene *A*, and $q (= 1 - p)$ is the frequency of the gene *a*, and c_T , c_O , d_T , and d_O are as previously defined. If the putative and "true" fathers and the mother are unrelated, then

$$c_o = d_o = 1; c_T = d_T = 0; \text{ and}$$

$$\frac{D}{\lambda} = pq^4. \quad (6)$$

The arguments here given for a single pair of autosomal alleles can be extended, of course, to multiple autosomal or to sex-linked alleles. To attempt to do so exhaustively, in the former instance, is difficult. As Cotterman (1953) has shown, there are no less than 52 regular three-allele systems, and, since in general, no two have the same statistical properties each requires a separate treatment. On the other hand, an extension of these arguments to sex-linked loci is straightforward. First, we note that all nonpaternity of sons is cryptic; their X-chromosomes are derived from their mothers. Second, and with respect to daughters, to detect nonpaternity the "true" father must be genotypically unlike the putative father. It follows, therefore, that the putative father and "true" father must have no genes identical by descent. The mother and the "true" father may, however, have one gene identical by descent. We find the general expressions to be

$$\frac{D}{\lambda} = pq(1 + q)(d_oq + (1/2)d_T)$$

for the case of sex-linked alleles with dominance (e.g., the human Xg^a system), and

$$\frac{D}{\lambda} = pq[2d_o(1 - pq) + (3/2)d_T]$$

for the case of sex-linked alleles without dominance. In the event, the "true" father and mother are unrelated, these expressions reduce to

$$\frac{D}{\lambda} = pq^2(1 + q)$$

and

$$\frac{D}{\lambda} = 2pq(1 - pq)$$

respectively.

THE ESTIMATION OF λ AND p

To estimate λ and p we proceed as follows: We assume that a series of mother-child-putative father combinations are examined with respect to some trait, and that on the basis of this examination the child, or if you will, the combination, is assigned to one of a series of mutually exclusive categories. The latter recognize the putative father's genotype (or phenotype) and whether the child's genotype (or phenotype) is compatible with that of the putative father. Within a random sample of N combinations, suppose n_i mother-child-putative father groups fall into category i ($i = 1, 2, \dots, k$). It is assumed that the n_i are multinomially distributed with parameters N , λ , and p .

TABLE 2. THE DISTRIBUTION OF COMPATIBLE AND INCOMPATIBLE CHILDREN BY GENOTYPE OF PUTATIVE FATHER

PUTATIVE FATHER	CHILD			
	Compatible		Incompatible	Σ
AA	o	C_1	K_1	
	e	$N[p^2 - \lambda p^2 q(1 - pq)]$	$N\lambda p^2 q(1 - pq)$	Np^2
AB	o	C_2	K_2	
	e	$2pqN$	0	$2pqN$
BB	o	C_3	K_3	
	e	$N[q^2 - \lambda pq^2(1 - pq)]$	$N\lambda pq^2(1 - pq)$	Nq^2
	Σ	$N[1 - \lambda pq(1 - pq)]$	$N\lambda pq(1 - pq)$	N

o = observed number; e = expected number.

Alleles without Dominance

Consider, again, the case of a trait determined by a single pair of autosomal alleles without dominance. There exist six mutually exclusive categories, namely, putative father AA-child compatible (or incompatible); putative father AB-child compatible (or incompatible); putative father BB-child compatible (or incompatible). As previously remarked, one of these cells, putative father AB-child incompatible, is a null set. Table 2 gives expressions for observed and expected numbers of children compatible and incompatible with the genotype of the putative father. It should be noted that the two ways of classification, namely, genotype of putative parent and compatibility of child, are not independent. The partial sums in table 2 can not be multiplied, therefore, to obtain the expected numbers in each cell. We take as the "best" estimates of λ and p those values which maximize the likelihood of the observed array. The likelihood function is

$$L = \frac{N!}{C_1!C_2!C_3!K_1!K_2!K_3!} [p^2 - \lambda p^2 q(1 - pq)]^{C_1} [2pq]^{C_2} [q^2 - \lambda pq^2(1 - pq)]^{C_3} [\lambda p^2 q(1 - pq)]^{K_1} [\lambda pq^2(1 - pq)]^{K_3}$$

where $N = C_1 + C_2 + C_3 + K_1 + K_2 + K_3$. Differentiation of the logarithm of L with respect to λ and p leads to the expressions

$$\begin{aligned} \frac{\partial(\log L)}{\partial \lambda}: \quad & \frac{K_1 + K_3}{\lambda} - \frac{C_1 q(1 - pq)}{1 - \lambda q(1 - pq)} - \frac{C_3 p(1 - pq)}{1 - \lambda p(1 - pq)} = 0 \\ \frac{\partial(\log L)}{\partial p}: \quad & \frac{(2C_1 + C_2 + 2K_1 + K_3)}{p} - \frac{(C_2 + 2C_3 + K_1 + 2K_3)}{q} + \\ & \cdot \frac{(K_1 + K_3)(p - q)}{1 - pq} + \frac{C_1 \lambda (1 - 2pq + q^2)}{1 - \lambda q(1 - pq)} - \\ & \frac{C_3 \lambda (1 - 2pq + p^2)}{1 - \lambda p(1 - pq)} = 0. \end{aligned}$$

These equations may be solved iteratively for λ and p .

The variances of the estimates of λ and p may be calculated according to the equations

$$\sigma_{\lambda}^2 = \frac{I_{pp}}{\Delta}; \quad \sigma_p^2 = \frac{I_{\lambda\lambda}}{\Delta}$$

where Δ is the determinant of the information matrix,

$$\Delta = \begin{vmatrix} I_{\lambda\lambda} & I_{\lambda p} \\ I_{p\lambda} & I_{pp} \end{vmatrix} = I_{\lambda\lambda}I_{pp} - I_{\lambda p}^2$$

and

$$\begin{aligned} I_{pp} &= \sum_j \frac{1}{e_j} \left(\frac{\partial e_j}{\partial p} \right)^2 \\ I_{\lambda p} &= I_{p\lambda} = \sum_j \frac{1}{e_j} \left(\frac{\partial e_j}{\partial p} \right) \left(\frac{\partial e_j}{\partial \lambda} \right) \\ I_{\lambda\lambda} &= \sum_j \frac{1}{e_j} \left(\frac{\partial e_j}{\partial \lambda} \right)^2 \end{aligned}$$

In these expressions, e_j is the expected number in the j^{th} class.

A summary of the calculation of $\left(\frac{\partial e}{\partial \lambda} \right)$ and $\left(\frac{\partial e}{\partial p} \right)$ is presented in table 3.

The algebraic expressions for I_{pp} , $I_{\lambda p}$, and $I_{\lambda\lambda}$ are quite cumbersome, and are, therefore, not presented here. The variances can, of course, be readily calculated by substituting numerical values into the expressions for e , $\frac{\partial e}{\partial \lambda}$, and $\frac{\partial e}{\partial p}$.

Alleles with Dominance

Consider, now, the case of a trait determined by a single pair of autosomal alleles with dominance, say A and a . Again, as a convenience, we assume that the mother, "true" father, and putative father are unrelated. The likelihood function can be shown to be

$$L = \frac{N!}{C_1!C_2!K!} (1 - q^2)^{C_1} (q^2 - \lambda pq^6)^{C_2} (\lambda pq^6)^K$$

where C_1 is the number of children compatible with the dominant phenotype, C_2 the number compatible with the recessive phenotype, and K the number incompatible with the latter phenotype. When the logarithm of this function is differentiated with respect to λ and p , and the resulting equations set equal to zero, we have

$$\begin{aligned} \frac{2C_1q}{1 - q^2} - \frac{C_2[2 + \lambda q^4(q - 6p)]}{q(1 - \lambda pq^4)} + \frac{K(q - 6p)}{pq} &= 0 \\ \frac{K}{\lambda} - \frac{C_2pq^4}{1 - \lambda pq^4} &= 0 \end{aligned}$$

TABLE 3. THE EXPECTED NUMBERS AND PARTIAL DERIVATIVES
APPROPRIATE TO THE VARIOUS PUTATIVE FATHER-CHILD CLASSES

Putative father	Class	Child	Observed (o)	Expected (e)	$\frac{\partial e}{\partial \lambda}$	$\frac{\partial e}{\partial p}$
AA	Compatible	C ₁	$N[p^2 - \lambda p^2 q(1 - pq)]$	$-Np^2 q(1 - pq)$	$N[2p - \lambda(2p^2 q - 3p^2 q^2 + 2pq - p^2)]$	
AB	Compatible	C ₂	$2Npq$	O	$N[2q - 2p]$	
BB	Compatible	C ₃	$N[q^2 - \lambda pq^2(1 - pq)]$	$-Npq^2(1 - pq)$	$-N[2q - \lambda(2pq^2 - 3p^2 q^2 + 2pq - q^2)]$	
AA	Incompatible	K ₁	$N\lambda p^2 q(1 - pq)$	$Np^2 q(1 - pq)$	$N\lambda(2p^2 q - 3p^2 q^2 + 2pq - p^2)$	
AB	Incompatible	K ₂		O	O	
BB	Incompatible	K ₃	$N\lambda pq^2(1 - pq)$	$Npq^2(1 - pq)$	$-N\lambda(2pq^3 - 3p^2 q^2 + 2pq - q^2)$	
Total		N	N	O	O	

These equations may be solved directly to obtain

$$q = \sqrt{\frac{C_2 + K}{N}}$$

$$\lambda = \frac{KN^3}{(C_2 + K)^3 [N - \sqrt{N(C_2 + K)}]}$$

The variances of these estimates can, as before, be obtained from the relationships

$$\sigma_\lambda^2 = \frac{I_{pp}}{\Delta}; \quad \sigma_p^2 = \frac{I_{\lambda\lambda}}{\Delta}; \quad \sigma_{p\lambda} = -\frac{I_{\lambda p}}{\Delta}$$

where, now,

$$I_{\lambda\lambda} = \frac{Npq^6}{\lambda(1 - \lambda pq^4)}$$

$$I_{\lambda p} = \frac{Nq^5(q - 4p)}{1 - \lambda pq^4}$$

$$I_{pp} = N \left(\frac{4q^2}{1 - q^2} + \frac{(2 + \lambda q^4(q - 6p))^2}{1 - \lambda pq^4} + \frac{\lambda q^4(q - 6p)^2}{p} \right)$$

Finally, consider the case of a trait determined by a single pair of sex-linked alleles with dominance. For the situation in which the "true" and putative fathers and the mother are unrelated, the likelihood function is

$$L = \frac{N!}{C_1!C_2!K_1!K_2!} (q - \lambda pq^3)^{C_1} (p - \lambda pq^2)^{C_2} (\lambda pq^3)^{K_1} (\lambda pq^2)^{K_2}$$

where C_1 and K_1 are the numbers compatible and incompatible with the phenotype (aY), and C_2 and K_2 are the corresponding numbers for the phenotype (AY). If the expressions which are obtained from differentiating log L with respect to λ and p are equated to zero, we have

$$-C_1 p(1 + \lambda q^2(q - 3p))(1 - \lambda q^2) + C_2 q(1 - \lambda q(q - 2p))(1 - \lambda pq^2) + (K_1(q - 3p) + K_2(q - 2p))(1 - \lambda q^2(1 + p) + \lambda^2 pq^4) = 0$$

$$\lambda^2 Npq^4 - \lambda q^2(K_1 + K_2)(1 + p) - (C_1 + C_2 - C_1 q) + (K_1 + K_2) = 0$$

where $N = C_1 + C_2 + K_1 + K_2$. These equations may be solved for λ and p by iteration.

The variances of these estimates we obtain from

$$I_{\lambda\lambda} = Npq^2 \left(\frac{pq^2}{1 - \lambda pq^2} + \frac{q^2}{1 - \lambda q^2} + \frac{1 + q}{\lambda} \right)$$

$$I_{\lambda p} = \frac{Npq^2[1 - \lambda q^2(3p - q)]}{1 - \lambda pq^2} + \frac{Nq^2[1 + \lambda q(2p - q)]}{1 - \lambda q^2} - \frac{Nq^2(3p - q) - Nq(2p - q)}{1 - \lambda pq^2}$$

$$I_{pp} = \frac{N[1 - \lambda q^2(3p - q)]^2}{q(1 - \lambda pq^2)} + \frac{N[1 + \lambda q(2p - q)]^2}{p(1 - \lambda q^2)} + \frac{N\lambda q(3p - q)^2}{p} + \frac{N\lambda(2p - q)^2}{p}$$

TABLE 4. A SUMMARY OF THE CALCULATIONS LEADING TO ESTIMATES OF P AND λ

Putative father	Class	Child	Observed (o)	Expected (e)	$\frac{\delta e}{\delta \lambda}$	$\frac{\delta e}{\delta p}$	$\frac{1}{e} \left(\frac{\delta e}{\delta \lambda} \right)^2$	$\frac{1}{e} \left(\frac{\delta e}{\delta \lambda} \right) \left(\frac{\delta e}{\delta p} \right)$	$\frac{1}{e} \left(\frac{\delta e}{\delta p} \right)^2$
M	Compatible	Compatible	59	64.10	-23.87	250.02	8.889	-93.10	9,52
MN	Compatible	Compatible	129	121.21	0	-24.01	0	0	4,756
N	Compatible	Compatible	50	52.35	-21.61	-225.31	8.921	93.01	969.7
M	Incompatible	Incompatible	4	2.802	23.87	4.970	203.3	42.34	8,815
MN	Incompatible	Incompatible	0	0	0	0	—	—	—
N	Incompatible	Incompatible	1	2.539	21.61	-5.677	183.9	-48.32	12.69
Σ			243	243.0	0	0	$I_{\lambda\lambda} = 405.0$	$I_{\lambda p} = -6.07$	$I_{pp} = 19.1$

$$\frac{\delta(\log L)}{\delta \lambda} = \frac{5}{.1174} - \frac{59(.4753)(.7506)}{1 - (.1174)(.4753)(.7506)} - \frac{50(.5247)(.7506)}{1 - (.1174)(.5247)(.7506)}$$

$$= -0.02$$

$$\frac{\delta(\log L)}{\delta p} = \frac{256}{.5247} - \frac{235}{.4753} + \frac{5(.0494)}{.7506} + \frac{59(.1174)(1 - .4988 + .2259)}{1 - (.1174)(.4753)(.7506)} - \frac{50(.1174)(1 - .4988 + .2753)}{1 - (.1174)(.5247)(.7506)}$$

$$= -5.7$$

APPLICATION OF THE METHOD TO A WORKED EXAMPLE

In table 4 are presented unpublished data of Gershowitz on the distribution of M-N blood groups among Detroit Negroes. If p is the frequency of M, and $q = 1 - p$ is the frequency of N, then we take as preliminary estimates of p and q the values obtained by merely counting the M and N genes among the putative fathers, namely,

$$p_1 = \frac{(59 + 4) + (\frac{1}{2})(129)}{243} = 0.5247$$

$$q_1 = \frac{(\frac{1}{2})(129) + (50 + 1)}{243} = 0.4753$$

The substitution of these values into

$$\frac{\delta \log L}{\delta \lambda} = 0$$

leads to 0.1174 as the preliminary estimate of λ , say λ_1 . The estimates p_1 , q_1 , and λ_1 are used to compute numerical values for $\frac{\delta \log L}{\delta \lambda}$ ($= a$), $\frac{\delta \log L}{\delta p}$ ($= b$), $I_{\lambda\lambda}$, $I_{\lambda p}$, and I_{pp} . A summary of these calculations is set out in table 4. The equations

$$I_{\lambda\lambda}\Delta\lambda_1 + I_{\lambda p}\Delta p_1 = a$$

$$I_{\lambda p}\Delta\lambda_1 + I_{pp}\Delta p_1 = b$$

are solved simultaneously for $\Delta\lambda_1$ and Δp_1 . Thus

$$405.0\Delta\lambda_1 - 6.07\Delta p_1 = -0.02$$

$$-6.07\Delta\lambda_1 + 1971.\Delta p_1 = -5.7$$

from which

$$\Delta\lambda_1 = -0.00009 \text{ and } \Delta p_1 = -0.0029.$$

New estimates of λ , p , and q are formed according to the equations

$$\lambda_2 = \lambda_1 + \Delta\lambda_1$$

$$p_2 = p_1 + \Delta p_1$$

$$q_2 = 1 - p_2$$

The procedure is repeated until sufficiently small values for $\Delta\lambda$ and Δp are obtained. It is worth noting that this procedure lends itself readily to programming for a digital computer.

In the present instance, after the second iteration, we arrive at the values

$$\lambda = 0.1173$$

$$I_{\lambda\lambda} = 405.6$$

$$p = 0.5218$$

$$I_{\lambda p} = -5.41$$

$$q = 0.4782$$

$$I_{pp} = 1971.$$

Finally, for the variances of λ and p , we obtain

$$\sigma_\lambda = 0.002466$$

$$\sigma_p^2 = 0.000507$$

Thus, the interval defined by the estimate λ plus and minus twice its standard error proves to be 0.018 to 0.217.

The "goodness of fit" of the model to the data is subject to test. If, in the present instance, the observed and expected numbers are contrasted in the conventional χ^2 manner, we obtain $\chi^2 = 2.44$, which for two degrees of freedom is not significant at the 1 per cent level.

SUMMARY

A general expression is derived for the relationship which exists between the over-all frequency of nonpaternity (λ) and the frequency (D) which can be detected on the basis of examinations of mother, child, and putative father with respect to a particular trait. Certain special cases of particular interest are deduced from this more general frequency. A method is then presented for the estimation of the over-all frequency of nonpaternity; the method which is given also affords estimates of the frequencies of the genes associated with the trait which provides the basis for the paternal exclusion.

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Inheritance of Blood Group Antigens in a Largely Eskimo Population Sample

MARION LEWIS, BRUCE CHOWN AND HIROKO KAITA

Department of Paediatrics, University of Manitoba and Rh Laboratory, Winnipeg, Canada

THERE IS A PAUCITY of published data about the inheritance of antigens in a number of blood group systems, notably the Kell, Duffy, Kidd and Lutheran systems, and the Ss antigens of the MNSs system; even for the Rh system the data are not extensive. Further, most family studies have been on Caucasians. The data presented here are principally for Eskimos; there are a few Amerindians in population No. 5 of table 1. These data have been gathered over a period of years; details about all but one (No. 3 of table 1) of the population samples and the methods of testing them have been (Chown and Lewis, 1959; 1960; 1962) or will be published. The make-up of the total population sample is summarized in table 1, the inheritance in the various systems set out in tables 2 to 8.

The communities are largely inbred; near and distant relatives occur in the mating types. Where the genotype of the parents are not known, as in the ABO system, no attempt has been made to calculate the expected distribution in the

TABLE 1. POPULATION SAMPLES STUDIED

Location and Racial Make-up of Population	Matings	Children
1. Southampton Island (Chown and Lewis, 1960); north end of Hudson Bay. Eskimo and Eskimo-White hybrids. Only 18 families with 64 children typed in Duffy system; none in Kidd system.	19	66
2. Coppermine and Victoria Island (Chown and Lewis, 1959); North-West Territories. Almost pure-blood Eskimo; very little white admixture. Only 19 families with 48 children typed in Duffy system; none in Kidd system.	30	70
3. Kodiak Island, Alaska. Eskimo-White hybrid. Only four families with 13 children typed in Kidd system.	7	21
4. Aleutian Peninsula, Alaska. (Chown and Lewis, 1962). Eskimo-White hybrid. Only 18 families with 70 children typed in Kidd system.	31	115
5. Alaska Mainland. Mixed sample of Athapaskan Indians, Indian-White hybrids, Eskimos and Eskimo-White hybrids. Part of a study with Dr. Edward M. Scott, Arctic Health Research Center, Anchorage, Alaska, of possible linkage between a blood group locus and a recessive gene for methemoglobinemia. Only 33 families with 109 children typed in Kidd system.	43	144
Maximum total	130	416
For Duffy system	118	391
For Kidd system	55	192

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Postal address: 735 Notre Dame Ave., Winnipeg 3, Canada.

TABLE 2. THE ABO SYSTEM

Matings Type	No.	Total	O	A	Children B	AB
O x O	18	60	60	—	—	—
O x A	50	146	67	79	—	—
O x B	8	18	7	—	11	—
O x AB	3	19	—	14	5	—
A x A	33	115	18	97	—	—
A x B	13	43	1	16	7	19
A x AB	4	12	—	8	1	3
B x B	1	3	—	—	3	—
Total	130	416				

TABLE 3A. THE MNSS SYSTEM

Matings		Children							
Type	No.	Total	M		MN		N		χ^2 for 1 d.f.
			Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
M x M	28	91	91	91	—	—	—	—	
M x MN	50	149	77	74.5	70	74.5	2*	—	1.11
M x N	15	60	—	—	60	60	—	—	
MN x MN	23	74	21	18.5	37	37	16	18.5	0.68
MN x N	14	42	—	—	27	21	15	21	3.42
N x N	—	—	—	—	—	—	—	—	
Total	130	416							

*The two exceptions are a two year old and a one year old in a family of nine children. We had at the time some doubts that the information given about the children's parentage was correct. The other blood groups, however, do not rule out either putative parent.

TABLE 3B. THE MNSS SYSTEM

Matings		Children							
Type	No.	Total	S		Ss		s		χ^2 for 1 d.f.
			Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
S x S	2	3	3	3	—	—	—	—	
S x Ss	10	26	12	13	14	13	—	—	0.16
S x s	10	30	—	—	30	30	—	—	
Ss x Ss	18	60	22	15	29	30	9	15	5.70
Ss x s	48	160	—	—	91	80	69	80	3.02
s x s	42	137	—	—	—	—	137	137	
Total	130	416							

Sera used on all bloods: Anti-M, -N, -S, -s.

TABLE 4A. THE RH SYSTEM

Matings		Children							
Type	No.	Total	C		Cc		c		χ^2 for 1 d.f.
			Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
C x C	15	46	46	46	—	—	—	—	
C x Cc	27	96	54	48	42	48	—	—	1.50
C x c	18	56	—	—	56	56	—	—	
Cc x Cc	22	81	13	20.25	47	40.5	21	20.25	3.67
Cc x c	37	111	—	—	51	55.5	60	55.5	0.72
c x c	11	26	—	—	—	—	26	26	
Total	130	416							

Type abbreviations: C = C+c-; Cc = C+c+; c = C-c+.

Two samples in population 4 were C^wc; these are included in the Cc type.

TABLE 4B. THE RH SYSTEM

Matings		Children						χ^2 for 1 d.f.
Type	No.	Total	E		Ee		e	
			Obs.	Exp.	Obs.	Exp.	Obs.	
E x E	8	22	22	22	—	—	—	—
E x Ee	26	63	31	31.5	32	31.5	—	—
E x e	13	36	—	—	36	36	—	—
Ee x Ee	20	82	21	20.5	44	41	17	20.5
Ee x e	37	118	—	—	54	59	64	59
e x e	26	95	—	—	—	—	95	95
Total	130	416						

Type abbreviations: E = E+e—; Ee = E+e+; e = E—e+.

TABLE 4C. THE RH SYSTEM

Matings		Children		
Type	No.	Total	D+	D—
D+ x D+	126	406	396	10
D+ x D—	3	9	6	3
D— x D—	1	1	—	1
	130	416		

All but one of the D-negative persons were members of population 4.

TABLE 4D. THE RH SYSTEM

Matings		Children						χ^2 for 1 d.f.
Type	No.	Total	f+		f—			
			Obs.	Exp.	Obs.	Exp.		
f+(a) x f—	20	62	34	31	28	31		0.58
f+(b) x f—	3	8	8	8	—	—		
f+(a) x f+(a)	5	20	16	15	4	5		0.27
f+(a) x f+(b)	3	12	12	12	—	—		
f— x f—	50	177	—	—	177	177		
Total	81	279	70	66	209	213		

Type abbreviations: f+(a) = CcDe, CwcDe, cDEe and cEe. f+(b) = ce and cDe. f— = CDe, cDE, CcDEe, CDEe.

Sera used for Tables 4A — 4D.

Anti -C, -Ce, -C^w, -c, -D, -E, -e on all bloods. Anti -f(ce) for populations 3, 4 and 5 of Table 1.

TABLE 5. THE KELL SYSTEM

Matings		Children			
Type	No.	Total	k ^b	K ^b k ^b	k ^a k ^b
k ^b x k ^b	125	398	398	—	—
k ^b x K ^b k ^b	4	13	5	8	—
k ^b x k ^a k ^b	1	5	2	—	3
Total	130	416			

Sera used: anti -K, -k, -Kp^a, -Kp^b. Type abbreviations: k^b = K— k+ Kp(a—b+); K^bk^b = K+ k+ Kp(a—b+); k^ak^b = K— k+ Kp(a+b+).

offspring. However, where the genotypes of the parents are known and where the number of offspring seemed large enough (MN, Ss, Rh, Duffy and Kidd systems) the expected distribution has been calculated within each mating type. There are two significant discrepancies: an excess of S and a shortage of s children from Ss x Ss matings and an excess of Fy^b children from $Fy^a Fy^b$ x $Fy^a Fy^b$ matings. It is unlikely that either of these discrepancies can be accounted for by technical error; linkage with MN provides a check on the Ss results and only one exception (see Fig. 1) was found. Since both discrepancies occur in the mating type which allows of the greatest variation in the children we feel that they are probably related to sample size. The value of the data lies in further substantiating the current rules of inheritance in the systems studied.

Thirty-eight families with 115 children were tested with both anti-Lu^a and anti-Lu^b; all were Lu(a — b +). Some families were tested with anti-Mi^a, -Vw, -M^s, -He, -Be^a, -By^a, -Di^a or -V; all were negative. The numbers of matings and children so tested are set out in table 8.

A Possible Recombination in the MNSs System

Although there is no exception to the law of inheritance when the Ss antigens

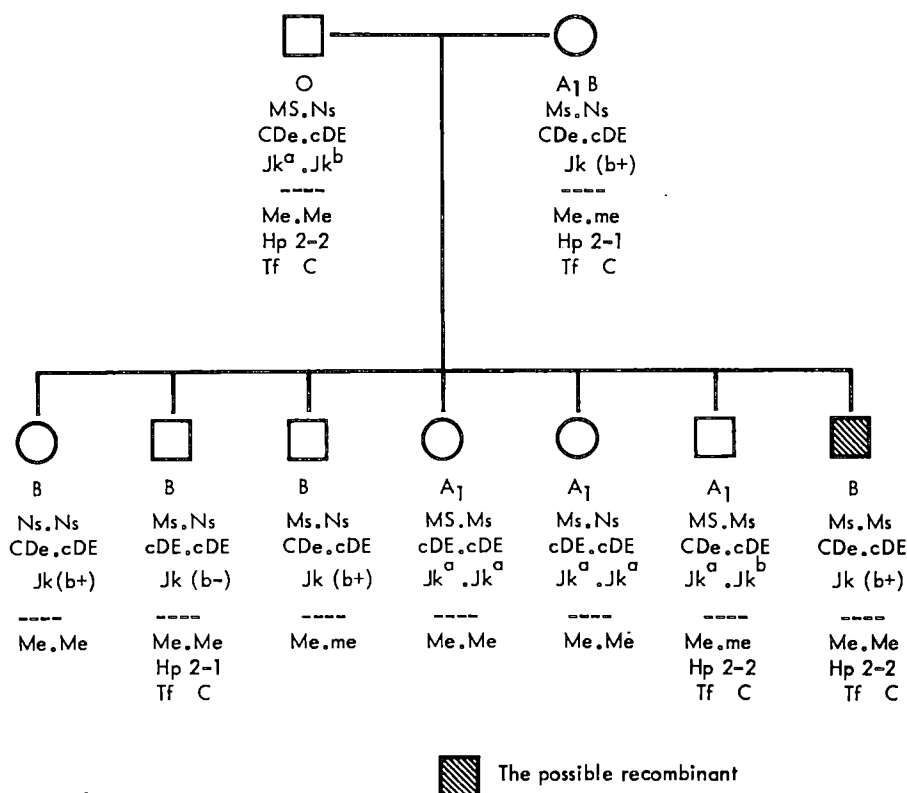


FIG. 1. A possible recombination in the MNSs system. All bloods P—; K— k+ Kp(a —b+); Fy(a+b—); Lu(a—b+); Mi(a—) Vw—; Be(a—); Di(a—); Wr(a—). Anti-Jk^a free of anti-B not available at the time. *me* = recessive gene for methemoglobin and *Me* the normal allele at this locus.

TABLE 6. THE DUFFY SYSTEM

Matings		Children							
Type	No.	Total	Fy ^a		Fy ^a Fy ^b		Fy ^b		χ^2 for 1 d.f.
			Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
Fy ^a x Fy ^a	51	175	175	175	—	—	—	—	0.32
Fy ^a x Fy ^a Fy ^b	39	110	58	55	52	55	—	—	
Fy ^a x Fy ^b	6	28	—	—	28	28	—	—	
Fy ^a Fy ^b x Fy ^a Fy ^b	14	44	8	11	17	22	19	11	5.82
Fy ^a Fy ^b x Fy ^b	8	34	—	—	15	17	19	17	0.48
Fy ^b x Fy ^b	—	—	—	—	—	—	—	—	
Total	118	391							

Sera used: anti -Fy^a, -Fy^b. Type abbreviations: Fy^a = Fy(a+b—); Fy^aFy^b = Fy(a+b+); Fy^b = Fy(a—b+).

TABLE 7. THE KIDD SYSTEM

Matings		Children							
Type	No.	Total	Jk ^a		Jk ^a Jk ^b		Jk ^b		χ ² for 1 d.f.
			Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
Jk ^a x Jk ^a	3	7	7	7	—	—	—	—	1.52
Jk ^a x Jk ^a Jk ^b	16	53	31	26.5	22	26.5	—	—	
Jk ^a x Jk ^b	7	24	—	—	24	24	—	—	
Jk ^a Jk ^b x Jk ^a Jk ^b	15	64	20	16	33	32	11	16	2.59
Jk ^a Jk ^b x Jk ^b	12	36	—	—	13	18	23	18	2.78
Jk ^b x Jk ^b	2	8	—	—	—	—	8	8	
Total	55	192							

Sera used: anti -Jk^a, -Jk^b. Type abbreviations: Jk^a = Jk(a+b—); Jk^aJk^b = Jk(a+b+); Jk^b = Jk(a—b+).

TABLE 8. FAMILIES NEGATIVE WITH VARIOUS ANTISERA

Type	Matings	Children
Mi(a—)	86	256
Vw—	92	321
M ^g —	80	251
He—	30	70
Bc(a—)	92	322
By(a—)	30	70
Di(a—)	116	364
V—	99	303

are considered alone, there is one exception when MN and Ss are considered together. The blood groups, serum types and methemoglobin genes of the family in which this occurred are given in Fig. 1. There is the possibility that during meiosis a cross-over occurred between the father's M and S and his N and s, the resulting chromosome carrying Ms being transmitted to his youngest child.

Recombination is a possibility, but illegitimacy must be considered more probable, even though the other blood groups, the serum factors, which were kindly determined by Dr. Eloise Giblett, and the methemoglobin genes do not exclude the husband as the possible father. Dr. Scott examined the husband and the child for small congenital anomalies which, had they been inherited by the child, might have weighed in favor of the child's legitimacy, but found none.

Palm prints of the husband and the child did not, according to Dr. Irene Uchida, Geneticist to the Children's Hospital, have any certain anomaly of pattern in common.

In a case reported by Shapiro (1956) a cross-over between hypothetical loci for MN and Ss, MN and He, or Ss and He during oögenesis could have accounted for an apparent anomaly of inheritance in the MNSs system but he thought illegitimacy a more likely explanation.

DISCUSSION

The population reported here is predominantly Mongoloid, although some of the subsamples have a considerable Caucasian admixture. The pattern of inheritance of the antigens tested for does not differ from that in Caucasians. If "silent genes" such as S^u, D⁻, K^o, Lu, Fy or Jk, or allelic antigens other than those examined for, occur in the population they do so in such low frequencies that their presence is not demonstrable in the families tested.

SUMMARY

The inheritance, in a largely Eskimo population, of antigens in the ABO, MNSs, Rh, Kell, Duffy, Kidd and Lutheran blood group systems and for several antigens not yet placed in known systems was found to conform to the pattern for their inheritance in Caucasians. No evidence was found for the occurrence of "silent genes" in such frequency as to disturb expectation significantly. A possible recombination in the MNSs system is recorded.

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A Genetic Study of Cleft Lip and Palate in Utah

CHARLES M. WOOLF, ROBERT M. WOOLF¹ AND T. RAY BROADBENT¹

Department of Zoology, Arizona State University, Tempe, Arizona

AN EXCELLENT genetics study on cleft lip and palate was carried out in Denmark by Fogh-Anderson (1942), and although the results were confirmed by studies in Canada (Fraser, 1955; Curtis, Warburton, and Fraser, 1961), there is need for large studies in other populations. No such study has been carried out in the United States. Cleft lip and palate appear to be etiologically heterogeneous (Fraser, 1955) and comparative studies in different populations would be one way of evaluating the over-all importance of genetic and nongenetic components. The purpose of the present paper is to report the results of a genetic study of congenital clefts of the lip and palate in Utah. The results of an analysis of certain nongenetic variables will be reported later. This study is part of a general investigation of various types of congenital malformations in Utah, taking advantage of a population that is predominantly Mormon and therefore relatively stable in the area. Strong family-ties and an interest in genealogical records facilitate genetic research among the Mormon people.

INCIDENCE OF CLEFT LIP AND PALATE

No data have been published on the incidence of congenital clefts of the lip and palate in any segment of the Utah population. Nursery records on file in the Latter-day Saints Hospital in Salt Lake City, Utah, were used to determine the incidence among live births occurring in the hospital during the years 1951-1961. The great majority of patients entering the hospital are Caucasian. This hospital is the largest in the state of Utah. About 23 per cent of the total births in the state occurred in this hospital during 1951-1961. When a newborn infant is taken into the nursery an entry is made in a record book of any observed congenital malformation. During the period 1951-1961, a total of 59,650 infants were registered in the nursery. A cleft of the lip or palate was recorded for 90 of these infants, giving an observed incidence of 1 in 662 (1.51 per thousand live births). The 90 cleft cases were classified as follows: cleft lip, 24; cleft lip and palate, 50; isolated cleft palate, 16.

Even though all cleft lip and cleft lip and palate cases might have been detected, isolated cleft palate cases could have been overlooked. Among a sample of surgical cases in Utah (see table 1), the numbers of cleft lip, cleft lip and palate, and isolated cleft palate cases approximate a 1:2:1 ratio. Fogh-Anderson (1942) has concluded that the real figures for the three groups at birth in Denmark are very close to 25 per cent, 50 per cent, and 25 per cent. Using the 1:2:1 ratio as a guide, allowance for missed isolated cleft palate cases can

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¹508 East South Temple, Salt Lake City, Utah

TABLE 1. CLASSIFICATION OF PROPOSITI

Type of Cleft	Number	%	% Female	% Male
Cleft Lip	128	23.1	36.7	63.3
Cleft Lip and Cleft Palate	290	52.5	26.9	73.1
Isolated Cleft Palate	135	24.4	52.6	47.4
Total	553			

be made by increasing the number from 16 to 24, which is equal to the number of cleft lip cases. This gives an adjusted total number of 98 cleft cases, and an adjusted incidence of 1 in 609 (1.64 per thousand live births). The adjusted incidence is probably closer to the true incidence among the 59,650 live births.

PROPOSITUS-STATISTICAL GENETICS STUDY

The names of 1,116 cleft cases were obtained from surgical records made available by physicians in the state of Utah. A total of 317 were from areas inconveniently located from Salt Lake City, Utah, and were removed from the study. With the aid of city directories, telephone books, and church records, an attempt was made to obtain a current address for each of the remaining cases. This method failed to locate 243 cases. The homes of the remaining 556 cases were then visited and one or more of the family members (usually the mother of the cleft case) was interviewed in order to obtain information of epidemiological interest. The study required extensive traveling throughout the state of Utah and adjacent regions of Idaho, Nevada, and Wyoming. Cooperation on the part of the family members was excellent. Although some were hesitant to supply information when first contacted, complete refusal to cooperate was only encountered in the relatives of three cleft cases. Upon completion of the study, information was available on the families of 553 cleft cases.

During the ascertainment of cleft cases and family histories, clefts of the lip and palate were considered as one group. Only upon completion of the study were the cases classified as cleft lip (symbolized by CL), cleft lip and cleft palate (symbolized by CLCP), and isolated cleft palate (symbolized by CP). No selection was made for or against cleft cases with other anomalies. Some of the propositi were already deceased when the family history was obtained.

The classification of the 553 cleft cases (propositi) as to type of cleft is shown in table 1. Even though cleft lip and cleft palate occur more often together than separately, the frequency of the three types in the sexes is one type of evidence (Fogh-Anderson, 1942) that isolated cleft palate is etiologically distinct from cleft lip with or without cleft palate. Both CL and CLCP occur more frequently in males than females, while CP is more common in females.

Evidence that CP is a genetically distinct disorder from CL and CLCP comes from a study of the frequency of these disorders in the relatives of the propositi. Information was obtained on the following classes of relatives: siblings, parents, children, grandparents, aunts, uncles, nieces, nephews, and first cousins. The data are summarized in table 2. The frequency of CL and CLCP is appreciable in the relatives of both the CL and CLCP propositi, but the frequency of CP

in these relatives is small. The situation is reversed in the relatives of the CP propiiti: CL and CLCP occur at a low frequency, but the frequency of CP is appreciable. This phenomenon can be studied objectively by comparing the observed percentage of cleft cases at birth among the living and deceased relatives of the propiiti with expected percentages derived from the nursery record data on file in the Latter-day Saints Hospital. For the CP the adjusted percentage is used. It is assumed here that the percentage of cleft cases among live births in this hospital represents the percentage occurring among live births in the general population. Since this assumption may not be justified the derived values represent only crude expected (risk) values for the general population.

Among the relatives of the propiiti with CL there is a significant increase in the frequency of both CL and CLCP, but not CP, over the frequency expected in the general population. The same situation prevails for the relatives of the CLCP propiiti. Among the relatives of the CP propiiti, CL and CLCP are present at the level of frequency expected in the general population, but the frequency of CP is increased significantly.

These data point out that CL and CLCP should be considered as one disorder, which can be symbolized by CL(P). This is done in table 3. The observed frequencies of CL(P) and CP in the relatives of the CL(P) and CP propiiti are compared with expected frequencies. It is clear that among the relatives of the CL(P) propiiti, there is a significant increase of CL(P), but CP is occurring at a frequency expected in the general population. Among the relatives of the CP propiiti there is a significant increase of CP, but not of CL(P).

TABLE 2. TYPES OF CLEFTS IN RELATIVES* OF PROPOSITI

Propiiti	Total No. of Relatives	Cleft Lip		Relatives: Cleft Lip and Palate		Isolated	
		No.	%	No.	%	Cleft Palate No.	%
Cleft Lip							
Observed	5,726	20	0.349	28	0.490	2	0.035
Expected†		2.3	0.040	4.8	0.084	2.3	0.040
Cleft Lip and Palate							
Observed	13,585	42	0.309	82	0.604	6	0.044
Expected†		5.5	0.040	11.4	0.084	5.5	0.040
Isolated							
Cleft Palate							
Observed	5,863	4	0.068	2	0.034	27	0.460
Expected†		2.4	0.040	4.9	0.084	2.4	0.040

* See text for classes of relatives.
† See text for expected values.

TABLE 3. TYPES OF CLEFTS IN RELATIVES* OF PROPOSITI

Propiiti	Total No. of Relatives	Cleft Lip With or Without		Relatives: Cleft Lip With or Without		Isolated	
		Without Observed	Cleft Lip With or Without Expected†	Cleft Lip With or Without Observed	Cleft Lip With or Without Expected†	Cleft Palate Observed	Cleft Palate Expected†
Cleft Lip With or Without							
Cleft Palate	19,311	172	24.0	8	0	7.8	
Isolated							
Cleft Palate	5,863	6	7.3	27	2.4		

* See text for classes of relatives.
† See text for expected values.

The frequency of CL(P) in the various classes of relatives of the propositi with CL(P) is shown in table 4. The observed percentage in each class is increased over the percentage found in the general population. The frequency of CP in the various classes of relatives of the CP propositi is shown in table 5. Again, when the number of relatives in a given class is large enough to be meaningful, the observed percentage is larger than the percentage found in the general population.

DISCUSSION

If two populations exhibited about the same incidence of congenital clefts of the lip and palate but differed in the frequency of responsible genetic and nongenetic factors, studies similar to the present one in each population might demonstrate the differences. For example, in the population where nongenetic factors were of prime importance, fewer familial aggregations of cases might be noted. The results of the present study and those carried out in Denmark and Canada are remarkably similar, suggesting a similarity of etiological factors in the populations. It would be informative to compare the results of studies carried out in low and high incidence populations.

The results of the present study support unequivocally the conclusions of Fogh-Anderson (1942) that CL and CLCP are due to the same genetic mechanism, which is different from the one giving rise to CP. The lip develops during the fifth to eighth week of gestation and the palatal region about the ninth week (Canick, 1954). A mechanism altering the first developmental process may secondarily affect the second; but the second process may be altered

TABLE 4. FREQUENCY OF CLEFT LIP WITH OR WITHOUT CLEFT PALATE
IN THE RELATIVES OF PROPOSITI WITH THIS DISORDER

Relative	Total No.	No. of Cases	%
Siblings	1,410	65	4.61
Parents	836	17	2.03
Children	164	7	4.27
Grandparents	1,715	6	0.35
Aunts and Uncles	4,164	29	0.70
Nieces and Nephews	832	7	0.84
First Cousins	10,240	41	0.40
Unrelated (General Population)			0.12

TABLE 5. FREQUENCY OF ISOLATED CLEFT PALATE IN THE RELATIVES
OF PROPOSITI WITH THIS DISORDER

Relative	Total No.	No. of Cases	%
Siblings	431	11	2.55
Parents	270	4	1.48
Children	46	4	8.70
Grandparents	540	1	0.18
Aunts and Uncles	1,267	4	0.32
Nieces and Nephews	162	0	0.00
First Cousins	3,147	3	0.09
Unrelated (General Population)			0.04

independently of the first. Although this genetic conclusion may be true in general, there is one noteworthy exception. In some families, fistula labii inferioris congenita is associated with cleft lip and palate. The mode of inheritance of the syndrome appears to be that of a single autosomal dominant gene (Van Der Woude, 1954). Some individuals with the gene may show CL, CP, and fistulae of the lower lip, while others possessing the gene may show a combination of two, one, or none of the traits. In these families CP may occur in the same sibship with CL or CLCP. This is evidence that in some cases a single genetic mechanism may interfere with either or both of the above mentioned developmental processes.

Evidence for the role of non-genetic factors in cleft occurrence comes from the observation of discordance for CL(P) and CP in the majority of MZ twins (Metrakos, Metrakos, and Baxter, 1958) and a slight maternal age effect for CL(P) (MacMahon and McKeown, 1953). The inducibility of clefts in experimental animals by vitamin deficient diets (Warkany, Nelson, and Schraffenberger, 1943; Nelson, Wright, Baird, and Evans, 1957), cortisone injections (Fainstat, 1954), and many other agents (Fraser, 1962), is perhaps further evidence that some clefts in man may have an environmental component. Although no viral agent has been definitely incriminated, Pleydell (1960) has reported a slightly increased incidence in urban areas as compared with rural areas in England, which may be attributable to a higher risk of infections in the more densely populated areas. This needs further testing.

All existing information suggests that CL(P) and CP are etiologically heterogeneous. In some cases a polygenic mechanism may be acting, perhaps of the type shown for polydactyly in guinea pigs by Wright (1934), while in others the genetic mechanism may be a single gene as shown for the syndrome consisting of fistulae on the lower lip and cleft lip and palate. Teratogenic agents interacting with genetic mechanisms would add further complexity to the etiology.

If CL(P) and CP can be determined by single genes, polygenes and non-genetic factors, then sporadic case families and multiple case families should be prevalent in a population. An attempt to demonstrate these families objectively can be made by comparing the frequency of families with 0, 1, 2, 3, 4 or 5 cleft cases, other than the proband, with expected frequencies assuming that the probability of a cleft in the other family members, for one or more reasons, is low, random, and uniform from family to family. In tables 6 and 7, the frequency of families showing 0, 1, 2, 3, 4, or 5 other cases of CL(P) and CP are compared with the Poisson distribution using the arithmetic means of the observed distributions. The observed and generated distributions are not homogeneous. For example, in table 6, it is noted that 305 of the families of the CL(P) probandi showed no other case of CL(P). The expected number is 277. At the other end of the distribution, three families showed four other cases where the expected number of families is 0.3, and one family showed five other cases where the expected number is 0.0. Although there is a slight bias in this analysis because multiple case families have the highest likelihood of being ascertained, the results suggest, nevertheless, that sporadic case families and multiple case families are occurring at an increased frequency. Similar conclu-

TABLE 6. DISTRIBUTION OF CLEFT LIP WITH OR WITHOUT CLEFT PALATE CASES IN THE FAMILIES OF 418 PROPOSITI WITH THIS DISORDER

No. of Cases of Cleft Lip With or Without Cleft Palate in Families (excluding propositi)	Observed No. of Families	Expected No. (Poisson Distribution) $\bar{x} = 0.4115$
0	305	277.0
1	75	114.0
2	22	23.5
3	12	3.2
4	3	0.3
5	1	0.0
6	0	0.0
Total	418	418.0

TABLE 7. DISTRIBUTION OF ISOLATED CLEFT PALATE CASES IN FAMILIES OF 135 PROPOSITI WITH THIS DISORDER

No. of Cases of Isolated Cleft Palate in Families (excluding propositi)	Observed No. of Families	Expected No. (Poisson Distribution) $\bar{x} = 0.200$
0	118	110.5
1	11	22.1
2	2	2.2
3	4	0.2
4	0	0.0
5	0	0.0
6	0	0.0
Total	135	135.0

sions are reached for the CP families (table 7). Consequently, CL(P) and CP may be strongly heritable in some families through the action of a dominant gene, less heritable in others through the interaction of polygenes and non-genetic factors, and may appear as phenocopies in still other families.

CONCLUSIONS

1. The incidence of congenital clefts of the lip and palate among 59,650 live births in the Latter-day Saints Hospital in Salt Lake City, Utah, was observed to be one in 662, or 1.51 per thousand live births.

2. The results of this study support the hypothesis that genetic components exist for these anomalies. A statistical analysis of the families of 553 propositi demonstrates that cleft lip (CL) and cleft lip with cleft palate (CLCP) have a genetic component in common, which is different from the one predisposing to isolated cleft palate (CP).

3. Sporadic case families and multiple case families occur at a frequency in the population supporting the hypothesis that congenital clefts of the lip and palate are etiologically heterogeneous.

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ACKNOWLEDGMENT

It is a pleasure to acknowledge the labors of the case workers, Mrs. Anna Beth Stucki and Mrs. Ruth Snow.

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Book Reviews

THE ORIGIN OF RACES. BY CARLETON S. COON. New York: Alfred A. Knopf. 1962. (724 pp., \$10.00)

In this impressive and controversial book, Dr. Coon traces the living races of man from their earliest appearance in the fossil record into the present. It is his contention that *Homo sapiens* evolved from a pre-existing species while retaining the same geographical races, and he marshalls a vast array of data in the attempt to support this thesis. The 32 plates, 84 drawings, 13 maps, 39 tables, voluminous footnotes, and 700 references attest the magnitude of the undertaking.

In the first three chapters the author considers general principles of evolution and their application to man, citing the findings of genetics, geography, geology, and climate in relation to man's structure and his adaptation to the environment. He explores the growth of human society and the interplay of physical and cultural factors in its development. The succeeding five chapters carry the story of evolution from the earliest primates through the earliest hominids, and the next four chapters trace the development of each of the major races.

Five races are recognized by Coon: Caucasoid, Mongoloid, Australoid, Congoid (Negroes and Pygmies of Africa), and Capoid (Bushmen and Hottentots). It is possible that this somewhat arbitrary grouping delineates the races more sharply than they exist in nature, thereby introducing an unconscious bias in favor of Coon's hypothesis.

To follow the author's argument one must distinguish between grades and lines of organisms. "A grade . . . is a stage of physical adaptation to a special way of life . . . A line is a lineage, a genetic continuum, a succession of animals in process of phyletic evolution. . . ." (p. 306). The Australopithecines, who occupied a wide territory from Africa to Java and China at the end of the Lower Pleistocene, may be considered one grade. *Homo erectus*, who had everywhere evolved from and replaced them by the Middle Pleistocene, may be considered a second grade; while *Homo sapiens* is regarded as a third. The lines correspond to the five races, each of which passed through each grade, but at a different time.

The Australoid line from the Pithecanthropi and Solo (all *erectus*), through Wadjak, Keilor, Talgai, and Cohuna (all *sapiens*), to the present race constitute the best documented sequence; its outline was evident to Weidenreich when he advanced his scheme of human evolution from which Coon borrows with full acknowledgement. The Mongoloids are said to stem from grandfather *Sinanthropus* (*erectus*), through a series of *sapiens* forms, most of them known only since 1955. In the Caucasoid line are Heidelberg (probably *erectus*), and a host of later finds (all *sapiens*). Africa remains the "Darkest Continent" in our knowledge of racial origins, some gaps remaining in the Capoid line and more in the Congoid. Ternifine and later finds in North Africa are seen as the

probable erectus grade of the Capoids, who subsequently migrated to their present home in South Africa, under pressure from advancing Caucasoids. The earliest Congoid erectus grade would be Chellian 3 recently found at Olduvai Gorge, followed in the Upper Pleistocene by Saldanha and Broken Hill (both considered erectus) and more recently by Asselar in the Sahara Desert (the earliest sapiens form). The Negroes supposedly crossed the "erectus-sapiens threshold" considerably later than the other races of mankind: "the Congoid line started on the same evolutionary level as the Eurasiatic ones in the Early Middle Pleistocene, and then stood still for a half million years," (p. 658). The admittedly scanty fossil evidence in the ancestry of the Negro, plus the debatable classification of Rhodesian man as clearly erectus, make this view unproved at best. Even if one race did indeed cross the sapiens threshold later than others, this need not indicate innate inferiority of that population at present. It can be argued that the ability to advance so far in the shorter period of time of "sapienshood" is testimony to the biological capability of that race. The claim that "Racial intermixture can upset the genetic as well as the social equilibrium of a group. . ." (p. 661) rests upon even less evidence.

In citing as the prevailing view, contrary to his own, the idea that *Homo sapiens* arose in Europe and Western Asia about 35,000 B.C. and spread over the world, Coon may be erecting a straw man. Surely, since the dating of Swanscombe and the interpretation of Steinheim and Ehringsdorf as possible sapiens forms, not to mention the significance of such debatable finds as Kanam and Kanjera, few authorities would take such an extreme position. It is likely that considerable gene flow occurred back in erectus times as well as in sapiens history, a view implied by Weidenreich's diagonals connecting fossil men in his well-known scheme in which verticals show race and horizontals depict grade.

Dobzhansky has expertly criticized the population genetics of Coon's book (*Scientific American*, February 1963, pp. 169-172). He points out that the possibility that the genetic system of living men could have independently arisen five times or even twice is vanishing small; the whole species is eventually transformed into a new species. Coon's major inconsistency is the view that *Homo erectus* and *Homo sapiens* were contemporaneous and apparently genetically isolated for 200,000 years; yet the modern descendants of *Homo sapiens* are not so genetically isolated. "For a single species to have arisen from two species that could not interbreed would indeed be extraordinary."

Frequently in this work cultural and biological processes appear to be confused. This is especially true in the opening chapter where the argument is advanced that the myriad unrelated languages of the world and the persistence of the stone age culture of the Australian aborigines into modern times disprove a relatively recent origin for the races of mankind (pp. 4-5). Elsewhere (p. 332), the claim is made that those races who "got fire before others did. . . were the first to receive its evolutionary benefits, and that those who obtained it last must have been correspondingly retarded."

A few factual errors, especially in anatomy, creep into the text and may make one question the over-all accuracy and the thoroughness of scholarship.

Whether or not one is convinced by the author's argument, he can read this work with pleasure and profit. The pleasure derives from Coon's lively style,

often laced with humor, and his willingness to indulge in original and thought-provoking speculations. The profit results from the selection of so much information from diverse fields bearing on human evolution, and from the careful description of a wealth of fossil material, some of it recently discovered, hitherto inadequately reported, or quietly reburied in inaccessible journals. The obvious danger in this work is that it is subject to misuse by the racist to be subverted toward an end which the author probably does not intend.

W. S. POLLITZER

*Department of Anatomy
University of North Carolina
Chapel Hill, North Carolina*

EXPANDING GOALS OF GENETICS IN PSYCHIATRY. EDITED BY FRANZ J. KALLMANN. New York: Grune and Stratton Co., 1962. (275 pp. \$6.75.)

This is a record of the special symposium held at the New York State Psychiatric Institute in October 1961, commemorating the 25th anniversary of that Institute's Department of Medical Genetics. It stands as a lasting tribute to the pioneering work of Dr. Kallmann, who founded the department in 1936, and who has himself perhaps contributed more than any other individual in this country to the field of psychiatric genetics.

The book is divided into five sections. The first four include behavioral and psychiatric genetics, progress in basic genetics, genetics of neurological disorders, and medical genetics in the field of mental health. Finally, the fifth section reports the testimonial and award speeches which highlighted the banquet. This section includes several pages of unposed and uncaptioned photographs in which appear many faces familiar to psychiatrists and/or geneticists.

Certainly, any effort must be lauded which seeks to bring together these widely divergent fields. Moreover, any meeting between the two disciplines must of necessity lack some coherence, for the simple reason that there is as yet little real application of the one to the other. The field of behavior genetics was unfortunately but little represented in the present symposium. Additionally, it seems doubtful that an awareness of basic mechanisms in genetics, such as the physicochemistry of DNA replication, can be of more than academic interest to the majority of psychiatrists at this time. And for those in psychiatry who are genetically oriented, most of the material presented here is available in standard publications.

In summary, *Expanding Goals of Genetics in Psychiatry* provides the uninitiated reader a few exciting glimpses of certain of these goals. But it mainly provides ample testimony that, just as the pathway between gene and behavior is long, tortuous and relatively untracked, so also is the pathway between genetics and psychiatry.

CHARLES R. SHAW

*Hawthorne Center
Northville, Michigan*

MONOZYGOTIC TWINS BROUGHT UP APART AND BROUGHT UP TOGETHER.
By JAMES SHIELDS. London: Oxford Univ. Press, 1962. (264 pp., \$11.50.)

Man's study of himself has always proved a strong attraction, although he tends to be a frustrating subject of investigation. This contumaciousness is frequently encountered in studies of the inheritance of physical traits and, when personality or behavior is to be explored, is likely to create a situation that may well be described as one where "geneticists fear to tread."

Notwithstanding the difficulties, the author of this book set forth to investigate the effect of inter-family differences in early childhood milieus on certain personality and behavioral traits, as well as on intelligence, weight and height.

A series of 88 pairs of identical (MZ) twins was used "to test the effects of the environment" on individuals with the "same hereditary equipment." One-half of them (15 female and 29 male sets) represented pairs *separated* (over 50 per cent before the age of 3 months). They were raised in different English homes for varying periods of time (five years onward) and usually reunited after leaving school. The remaining 44 MZ pairs comprised the *control* group, in which both twin partners lived in the same home and were matched for age (8-59 years) and sex. In addition, 32 pairs of two-egg (DZ) twins (11 separated and 20 controls) were included to supplement the findings. Zygosity was ascertained through analysis of blood groups, fingerprints, PTC tasting, color vision and the general principles of the similarity method.

Although many data were analyzed quantitatively, the author recognized "the complexity of genetical-environmental interaction and difficulty in assessing environmental and personality variables, together with the rarity of twins brought up apart." Hence, he did not really expect to obtain "results of great scientific precision." Nevertheless, some interesting findings were made.

A genetic component was reaffirmed in intelligence (by means of the Dominoes test and the Synonyms section of the Mill Hill Vocabulary Scale), and was also seen in the neuroticism and extroversion-introversion scores (derived from a self-rating "SRQ" questionnaire). While these scores were unaffected by differences in early family milieu, a significant intra-pair correlation was shown between high intelligence and early menarche. Also, the entire control group was found to be more extroverted than were the separated twins. However, early environment apparently influenced such traits as height and weight as well as the author's subjective assessment of personality resemblances.

Twins discordant for hysteria-like illnesses were observed in both the separated and the control groups, with several concordant pairs showing similar anxiety states and obsessional tendencies. One pair of separated MZ twins was classified as schizophrenic, with the same age of onset.

The author is careful in evaluating his findings, particularly when he attempts to appraise the role of early family settings or certain factors of selection, as well as the "environmental liability" of genetic traits. Yet, he may have been too categorical in assuming that "MZ have the same hereditary equipment, so there must be an environmental cause for any differences we find." No such clear distinction can be expected in view of the role played by expressivity, penetrance, somatic mutations, or mosaicism, in producing intra-pair variations. While this study unquestionably represented an exhaustive and energy-con-

suming undertaking, its over-all value was reduced by the lack of biochemical or physiological data. On the credit side are concise summaries of health, personality inventories, and intelligence test findings, as well as detailed descriptions of the events precipitating the early parting of the separated MZ and DZ twins.

DIANE SANK AND FRANZ J. KALLMANN
Department of Medical Genetics
New York State Psychiatric Institute
New York, New York

CLINICAL GENODERMATOLOGY. BY THOMAS BUTTERWORTH AND LYON P. STREAN *with an Introduction by James E. Wright, Jr.* Baltimore: Williams and Wilkins Co., 1962. (221 pp., \$13.50).

Butterworth and Strean have written the first definitive work in the field of dermatologic genetics since Cockayne's "Inherited Abnormalities of the Skin and Its Appendages" published in 1933 and now no longer in print. While Cockayne spent relatively little time describing the clinical condition and much time and labor searching the literature for illustrative pedigrees, Butterworth and Strean do almost the reverse.

In "Clinical Genodermatology," considerable space is devoted to definitions of the individual disease, its occurrence, symptoms, etiology, pathology, and treatment while there is only one short paragraph in which inheritance is discussed. No pedigrees are presented, only the way or ways in which the condition has been described as inherited, or presumably so. The authors admit their bibliographies are, of necessity, incomplete. An occasional one is somewhat out of date, such as the one on sickle-cell anemia.

Because of the brevity of the book, some descriptions seem slightly inadequate, except for the sophisticated reader. Such a one is that of Turner's syndrome. However, in practically all of the conditions discussed, excellent black and white photographs are most helpful. Included in the book are several conditions whose genetic determination is even questioned by the authors. Wright has written a fine, lucid, and simple introduction explaining the basic principles of genetics.

The entire book has been written in a straightforward, easy fashion which will be extremely simple for the dermatologist and not too difficult for clinicians in other disciplines to understand. For the latter, the dermatologic descriptions and photographs are more than adequate.

In brief, this book will be most helpful to the dermatologist, calling his attention to the many conditions in his specialty of genetic interest. For the non-dermatologist, whatever his approach to genetics may be, it will serve as an up-to-date grouping for him of the many skin conditions that are presently considered to have an hereditary background.

MAURICE T. FLIEGELMAN
1708 Heyburn Building
Louisville, Kentucky

Chromosomes in Medicine. Edited by JOHN L. HAMERTON. London: Medical Advisory Committee of the National Spastics Society in Association with Wm. Heinemann (Medical Books) Ltd. (232 pp. \$6.00.)

This collection of nine chapters on various aspects of human cytogenetics constitutes an excellent review of a field which is moving at break-neck speed. It is virtually impossible to keep abreast of the daily bulletins on human chromosomal abnormalities which appear in dozens of journals. This book serves as a spring-board for reference collectors and reviewers since all of the major contributions from 1959 through 1961 are included in the extensive bibliographies which occur at the ends of the chapters.

The clinician should be forewarned that the book is not a primer for the uninitiated. The cytologist will find little or nothing new, but the compilation of data in extensive tables is valuable. The contributors are renowned specialists in their respective fields: M. L. Barr, D. H. Carr, C. M. Clarke, C. E. Ford, M. Fraccaro, J. L. Hamerton, D. G. Harnden, P. E. Polani, R. W. Smithells, and N. D. Symonds. Topics covered include DNA, cell division, chromosome techniques and morphology, sex chromatin, sex chromosome and autosome anomalies, and a very complete review of the cytogenetics of mongolism.

(Margery W. Shaw)

An Introduction to the Cytogenetics of Polyploids. By G. W. P. DAWSON. Philadelphia: F. A. Davis Company, 1962. (96 pp. \$2.00.)

A glance at the index of this little monograph will convince the student of cytogenetics that a wealth of basic information has been packed into 90 pages of text. This book warrants careful reading, especially by those who would attempt to synthesize biologic concepts from cytologic observations and who lack thorough background in plant cytogenetics. The author writes lucidly and succinctly on such topics as pairing of chromosomes, mechanics of interchanges, cytogenetics of linkage, and behavior of trisomics, triploids, and tetraploids. He defines his terms with care and uses diagrams when indicated. It is advisable to read with pencil and paper in hand, for sketches and computations help to organize and synthesize the material which is concisely written but extremely condensed.

(Margery W. Shaw)

Studies in Genetics: The Selected Papers of H. J. Muller.

Edited by H. J. MULLER. Bloomington: Indiana University Press, 1962. (618 pp., \$7.95).

Several of his former students provided the impetus for the commemoration of Professor Muller's 70th birthday by the compilation of a volume selected from his publications. At their request, Professor Muller selected 90 papers from among the 336 titles in his complete bibliography, and provided additional explanatory notes for certain of these. The earlier writings are more heavily represented than the more recent ones. The presentation is divided into nine sections by major topics, with 12 interesting papers in the section on human and general genetics. The volume is indexed in detail. While this will be of particular interest to Professor Muller's many friends, it will also be of interest in relation to the history of genetics and useful for graduate student reading. (C. N. H.)

Osteogenesis Imperfecta in Sweden: Clinical, Genetic, Epidemiological and Socio-Medical Aspects.

By GUNNAR SMÅRS, with Ragnar Berfenstam, co-author of socio-medical section. Stockholm: Svenska Bokförlaget, 1961 (240 pp., Kr. 26.00). (paper-bound).

Detailed findings are presented from a study of 274 persons with osteogenesis imperfecta in Sweden, of whom 190 were personally examined. Of the total cases, 147 were probands

and 127 were secondary cases, and 84 of the latter had died or emigrated before beginning of the study. The frequency of osteogenesis imperfecta in Sweden was estimated at about 4 per 100,000. The genetic analysis agrees with previous studies in attributing most, and perhaps, all, cases to a "dominant" gene in heterozygous state. In most pedigrees bone fragility occurred in association with blue sclerae. In three pedigrees, including nine affected in seven sibships, bone fragility occurred without blue sclerae in any member of these families. The author suggests that a different mutation might be responsible for the disease in families in which blue sclerae are consistently absent. Five sibships were found with two or more affected members and with normal parents. The author concluded that recessive inheritance is unlikely, and that the effect of the dominant gene may be occasionally completely suppressed. This finding is also in accord with previous studies. Apparently sporadic cases were attributed to new mutations, and it was shown that some of these later produced affected children. The mutation rate was calculated as between 0.7×10^{-5} and 1.2×10^{-5} . The socio-medical section includes much interesting information, and points out that patients even with a severe functional reduction often show surprising ability to adapt themselves to the community. The fertility of affected individuals was estimated to be about 60 percent of normal. (C. N. H.)

Blood Groups in Man. By R. R. RACE and RUTH SANGER. Philadelphia: F. A. Davis, 1962 (456 pp., \$10.00).

The new edition of the standard reference work on this subject will be welcomed by all who are concerned in any way with human blood groups. A large amount of new material has become available during the four years since the last edition. This has necessitated the rewriting of about two-thirds of the volume. Much new material has been added concerning the ABO, MNS, and Rh systems. The chapter entitled "Other Blood Groups" in the third edition has been replaced with four new chapters, and the Auberger system is included. The new sex-linked blood group system, Xg, has been given a full chapter. This work is certainly the most comprehensive and authoritative summary of this subject now available. (C. N. H.)

ANNUAL MEETING

AMERICAN SOCIETY OF HUMAN GENETICS

July 19, 20, 21, 1963

New York City, Hotel Americana in conjunction with

Second International Conference on Congenital Malformations,

°

July 15-19, 1963

Notes Towards an Epidemiology of Spontaneous Abortion

W. H. JAMES

*Department of Psychology, Dalhousie University
Halifax, Nova Scotia, Canada*

THIS PAPER will examine data relating to two aspects of the epidemiology of spontaneous abortion. First I shall use a form of analysis (James, 1961) to reassess the statistical evidence for the clinical notion that women segregate into abortion-prone and abortion-resistant groups. Second, I shall consider the relation of maternal age and birth order to spontaneous abortion.

Since the discussion in both sections depends on the thesis that women differ greatly in their propensity to abort (called here their "abortion-probability"), and since this has been recently disputed (Warburton, 1961; Warburton and Fraser, 1959), I shall present fresh evidence on this point. And since the additional fertility following an abortion is a point which will also be raised in both sections, some preliminary observations will also be offered on this topic.

VARIATION BETWEEN WOMEN IN ABORTION-PROBABILITY

Three different lines of argument will be used in this context:

- (1) Observed frequencies of women within a gravidity group who have had 0, 1, 2 . . . abortions do not follow binomial expectations;
- (2) The variance of the observed distributions is large, yet the results of adjacent pregnancies in an obstetric history do not correlate;
- (3) Empirical examination suggests that pregnancies following a series of abortions have a far higher probability of aborting.

The Sample

Warburton (1961) presented a good summary of the types of bias associated with the commoner sources of data on spontaneous abortion. She concluded that the best source is a random sample of women interviewed by a skilled interviewer. Through the courtesy of the Trustees of the Institute for Sex Research of Indiana University, I have been given permission to present and re-analyse some of their previously published data and to analyse previously unpublished data as well. The sample of women from which these data were elicited has been described elsewhere (Gebhard, Pomeroy, Martin and Christenson, 1958, pp. 11-23). The training of the interviewers and the very thorough efforts to gain rapport with the subjects are described by Kinsey, Pomeroy and Martin (1948, ch. 2). Although the representativeness of the sample is admittedly incomplete (Gebhard *et al.*, 1958, pp. 18-23), it seems unlikely to have affected those features of the sample to be reported.

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The sample was of 781 white women, each of whom:

- a) was 36 or more years old at the time of interview,
- b) had married by the age of 30,
- c) had never been to prison,
- d) had had at least one pregnancy.

This sample includes the 759 gravidae mentioned on p. 136 of Gebhard *et al.* (1959). The additional 22 women were interviewed after the manuscript for the book had been prepared for publication. I shall call the 781 women the ISR Sample.

TABLE 1. WOMEN IN THE ISR SAMPLE. FREQUENCIES OF WOMEN WITH THE GIVEN COMBINATIONS OF PREGNANCIES AND SPONTANEOUS ABORTIONS

		Spontaneous abortions								Total	
		0	1	2	3	4	5	6	7	8	
P r e g n a n c i e s	1	136	27								163
	2	214	34	6							254
	3	98	45	15	5						163
	4	32	18	17	8	2					77
	5	8	11	9	3	2	0				33
	6	4	3	6	4	4	2	0			23
	7	0	1	1	0	1	2				5
	8						1				1
	9	1									1
	10	1			1		1	1	1		5
	11							1			1
	12						1			1	2
											728

The remaining 53 women in the ISR Sample had their every pregnancy terminate in induced abortion.

Table 1 shows the frequencies among these women with the given combinations of pregnancies and spontaneous abortions. In all cases induced abortions (therapeutic or criminal) are ignored; so, for example, a woman reporting one live birth, one spontaneous abortion and one induced abortion is recorded as having had two pregnancies, one of which yielded a live birth and the other a spontaneous abortion. It is here assumed that among those pregnancies which in fact ended in induced abortion, the same proportion would have spontaneously aborted if the pregnancies had not been artificially terminated. (Data on the gestation periods of illegal abortions are not known to this author.)

1. Testing for Binomial Expectations

If the probability of aborting a pregnancy were equal in all women and equalled $q = 1 - p$, then within a group of k women each of whom has had exactly n pregnancies, the expansion of the binomial $k(p+q)^n$ should give the expected frequencies of women who have had 0, 1, 2, . . . n abortions (this formulation is subject to a condition to be stated later). Table 2 shows the frequencies observed and those expected on the binomial hypothesis for those women with from three to six pregnancies ($p = 1 - q$, the basis of the expected frequencies, was calculated separately for each gravidity group).

TABLE 2. WOMEN IN THE ISR SAMPLE WITH 3, 4, 5 AND 6 PREGNANCIES. FREQUENCIES OF WOMEN OBSERVED AND EXPECTED ON THE DOUBLE BINOMIAL, SKELLAM'S DISTRIBUTION AND THE NEGATIVE BINOMIAL

		Abortions						
		0	1	2	3	4	5	6
3 Pregnancies	O	98	45	15	5			
	D	98.4	44.0	16.0	4.7			
	B	88.5	59.9	13.5	1.0			
								Total
4 Pregnancies	O	32	18	17	8	2		77
	D	31.5	20.6	13.8	8.7	2.4		77.0
	B	21.5	32.3	18.2	4.5	0.4		77.0
	S	30.4	22.2	14.2	7.5	2.7		77.0
	N	28.1	26.2	14.1	5.8	2.0		76.2
5 Pregnancies	O	8	11	9	3	2	0	33
	D	11.5	8.3	5.5	4.6	2.5	0.6	33.0
	B	6.4	12.4	9.6	3.7	0.7	0.1	33.0
	S	8.0	11.1	8.3	4.1	1.3	0.2	33.0
	N	7.7	11.7	8.4	3.7	1.2	0.3	33.0
6 Pregnancies	O	4	3	6	4	4	2	0
	D	4.2	3.9	3.9	4.8	4.0	1.8	0.3
	B	1.3	4.7	7.3	6.1	2.8	0.7	0.1

O = observed.

D = double binomial.

S = Skellam's distribution.

N = negative binomial.

For the binomials, chi-square = 19.8173 with 4 d.f., $p < 0.001$

Thus the fit is completely unsatisfactory. However, it was just remarked that the binomial frequencies would be expected only if a certain condition were satisfied. The condition is that women do not selectively conclude their reproductive performance according to the 'score' of their obstetric history. I shall later present data which suggest that, in fact, this condition is not satisfied. Women, it will be shown, are more likely to engage in a further pregnancy if the last one aborted than if the last one yielded a live birth. At first sight, it might be argued that it is this fact which frustrates the binomial expectations. However, if there were underlying binomial frequencies which had been disturbed by this selectivity, the effect would be to reduce the frequencies of women with high proportions of aborted pregnancies; there would be fewer women in the tails of the distributions. Yet the observed frequencies of women with 50 per cent or more pregnancies aborting *exceeds* the frequencies expected on the binomial hypothesis. It is concluded that if there are underlying binomial frequencies, it is not this selectivity which has interfered with them.

2. The Variance of the Observed Distributions

For the binomial distribution, the mean is np and the variance npq , so it is noteworthy that in three of the four observed distributions (for gravidae 3 to gravidae 6) the variance exceeded the mean. It implies either that the women varied in their abortion probabilities or that there is a positive correlation between the outcomes of successive pregnancies. This latter alternative may be tested by applying a runs test. If there were such a positive correlation, the

number of "runs" of similar outcome within each obstetric history would be expected to be smaller than would be predicted on the basis of chance. However, the runs test is not very efficient; for a woman with two abortions it cannot discriminate at the 5 per cent level unless she has had 14 or more pregnancies. And in no case can the test discriminate at the 5 per cent level in a history of less than nine pregnancies. Women in these categories are comparatively rare; even more rare are such women who can remember the *order* of the outcomes of their pregnancies. However, it is possible to combine the results of a number of tests of significance; the composite result may be expressed in terms of a single probability. Fisher (1932) considered the case of k values of a probability

p_1, p_2, \dots, p_k . He showed that $M^2 = -2 \log_e \prod_{j=1}^k p_j$ is distributed as χ^2 with $\nu = 2k$ degrees of freedom.

Swed and Eisenhart (1943) have tabulated the probability of $P [u \leq u']$ where u' is the observed number of runs in a sequence composed of n objects of one kind and m of another (m and n taking the values of two or more). So for each woman who has had 2+ live births and 2+ abortions (and who remembers the order in which they occurred) a probability may be quoted that the number of runs would be equal to or less than the observed number. From data provided by the Institute for Sex Research it was found that there were 30 white nonprison women whose obstetric histories could be tested in this fashion (The histories of prison women and Negro women were not so tested because it was believed that in the case of women recovering from or contracting syphilis during their reproductive lives, the abortion-probability might fluctuate, thus violating an assumption of the runs test.)

From the data, it was found that $M^2 = 43.761$ with 60 d.f., a value which is clearly not significant. It is concluded that the outcomes of adjacent pregnancies do not correlate and that, therefore, the women varied in their propensity to abort.

3. *Empirical Data on Recurrence Rates*

I have published data elsewhere (James 1962; James 1963) which indicate that a woman who aborted her last two or three pregnancies has a far higher chance of aborting her next pregnancy. Bearing in mind the lack of correlation between the results of adjacent pregnancies, this again suggests that women do, indeed, vary greatly in abortion-probability.

It should be noted that the high variances in the observed distributions suggest that the abortion-probability remained relatively constant within each woman; if it had varied, that would have reduced the variance (Edwards, 1960). Accordingly, it is concluded that abortion probabilities do, in fact, vary from one woman to another, although not appreciably within a given woman. This, of course, is intended only as a generalization, and is not meant to deny that in a few cases abortions are associated with blood group incompatibility, and that the probability of iso-immunization increases with gravidity (Glass, 1949). During the rest of this report, I shall treat this point as if it were established; I shall refer to "abortion-prone" and "abortion-resistant" women, with the implication that women vary (though do not necessarily segregate) in this regard. It is important, therefore, to consider how this probability is distributed.

ADDITIONAL FERTILITY FOLLOWING AN ABORTION

First, it is necessary to examine the consequences of a point already mentioned, the additional fertility contingent upon an abortion. As far as I am aware, this has not previously been discussed; other workers (Harris and Gunstad, 1936; Warburton, 1961) and I (James, 1961) have been too engrossed with the possibilities of curve-fitting to notice that it may easily interfere with frequencies of women to be expected with given combinations of live births and spontaneous abortions.

Suppose that a family size of two were universally desired, and suppose further that contraceptive technique were flawless. Then (ignoring the small number of matings characterized by involuntary secondary sterility) it would follow that every mating in which the completed family size involved two pregnancies would, in fact, comprise two live births. This is because those matings in which one or both of the first two pregnancies aborted, would engage in a further pregnancy, while those in which both pregnancies yielded live births would refrain from further reproductive efforts. In general, it would follow that every mating involving n pregnancies ($n = 2^+$), would involve $n-2$ abortions. Of course, neither of the conditions outlined is perfectly observed, but while they operate at all, it would be expected that those matings involving n pregnancies ($n = 3^+$), would be characterized by a reduction in variance.

It is, therefore, necessary to examine the extent to which these conditions *are* operating to see how much they may be expected to modify variance in this fashion.

1) It seems accepted that there is a general tendency for couples to think that a two-child family is the best size (Kiser and Whelpton, 1958), although it must be admitted that completed family size does not correlate highly with family size preference stated at the time of marriage (Westoff, Mishler and Kelly, 1957).

2) The efficiency of contraceptive technique was studied by Pearl (1932) who proposed the formula $R = \frac{\text{Total number of conceptions}}{\text{Total months of exposure}} \times 1200$ as a

measure of conception rate. In a study by Westoff, Potter, Sagi and Mishler (1961) it was found that of a sample of 1,165 white couples in seven of the largest cities in the U. S., 917 (79 per cent) had used contraceptive methods (of various sorts) for an aggregate of 28,607 months. The number of accidental pregnancies was 534, giving a failure rate of $R = 22.4$ conceptions/100 years of exposure. In contrast, it has been estimated (Tietze, 1959) that in the absence of contraception, R is of the order of 80 conceptions/100 years of exposure. So it may be taken as established that in contemporary America, contraception is extremely prevalent and relatively efficient. The effect it has on the relation between abortion rates and completed gravidity size is exemplified forcibly in the admittedly unrepresentative sample of Reed and Kelly (1958). The women in this sample had volunteered to participate as subjects in a longitudinal study of marital compatibility and were selected simply for their willingness to cooperate in the study. (I call them unrepresentative only because their mean intelligence was above average and because their use of contraceptive techniques was attended with more than usual success.) Among Reed and

Kelly's 2-gravidae, the incidence of abortion is 5 per cent. Among the first two pregnancies of their 3-gravidae, the comparable figure is 17 per cent (ignoring induced abortions and counting stillbirths as spontaneous abortions).

If it be accepted that selective application of contraception is responsible for this, it would follow that standard curves fitted to such data could form only the basis for tenuous conclusions. For unless one could estimate the extent to which the frequencies had been disrupted, one could make no firm inference even if one were able to fit the distributions.

However, this line of argument would seem applicable only when the abortion rate in families of n -gravidae is markedly less than in the first n pregnancies of $(n+1)$ -gravidae.

TABLE 3. PREGNANCIES OF WOMEN IN THE ISR SAMPLE.
FOR GRAVIDAE- $(n+1)$, THE ABORTION RATE WITHIN THE FIRST n
PREGNANCIES. CONTRASTED IS THE ABORTION RATE AMONG ALL
PREGNANCIES OF THE CORRESPONDING n -GRAVIDAE

	n			
	2	3	4	5
Abortion rate In the first n pregnancies of $(n+1)$ -gravidae	16.2	29.4	28.0	37.5
Abortion rate among all pregnancies of n -gravidae	9.1	18.0	27.0	28.0

In all cases, percentages are for totals of more than 100 pregnancies.

Table 3 makes this comparison for families in the ISR sample for values of n from $n=2$ to $n=5$. The rate in the first two pregnancies of 3-gravidae is almost double the rate in 2-gravidae. For other n , it may be noted that although the rates are always higher in the first n pregnancies of $(n+1)$ -gravidae than the rates in n -gravidae, the proportional difference in no other case is so great. So in these cases, the effect of additional fertility after abortion will be ignored for purposes of curve-fitting.

TESTING FOR SEGREGATION OF ABORTION PROBABILITY

It was proposed to test for segregation of abortion-probability by examining the fit to the double binomial $\nu_1 (p_1 + q_1)^n + \nu_2 (p_2 + q_2)^n$ (James, 1961). For each gravidity group, q_1 and q_2 were estimated, and then estimates of q_1 and q_2 for the whole sample were derived by weighting the separate estimates according to the number of women in the groups from which the estimates were made. Lastly, these over-all estimates of q_1 and q_2 were used to estimate ν_1/ν_2 separately for each gravidity group. The rationale of this was that if the selectivity mentioned were very powerful, then, of course, the fit could not be expected to be good. However, if it were weak, then for each gravidity group the ratio ν_1/ν_2 (i.e., the ratio of abortion-prone to abortion-resistant women in the gravidity group) would be expected to vary from group to group, increasing with gravidity. Then, within each gravidity group, the frequencies of women with 0, 1, 2, ..., n abortions should approximate to the sums of the appropriate terms

of the double binomial. The data for 2-gravidae were discarded as showing too much evidence of the selectivity. For the sample of 3, 4, 5 and 6-gravidae, the weighted estimates of q_1 and q_2 were 0.530949 and 0.104352, respectively, for the abortion-prone and abortion-resistant women (where $q = 1-p$ is the probability of aborting a pregnancy).

The same gravidity group frequency distributions of abortions were also fitted to (1) negative binomials to test the hypothesis that abortion-probability is a unimodal positively skewed variable (Greenwood and Yule, 1920) and (2) Skellam's distribution (Skellam, 1948; Edwards, 1958) to test the hypothesis that abortion-probability is a β -variate. (With small variance, at any rate, the β - and normal distributions are similar, and it is reasonable to test whether a biological parameter like p , with a small range of variation, may be nearly normally distributed.) For brevity this hypothesis will be referred to as the Skellam hypothesis. Table 2 shows the results of these fits.

Chi-square values were computed for all fits. In all cases where the expected frequencies were less than five, the cell values were pooled until this value was exceeded. The results were as follows:

For the double binomials, chi-square	= 6.893; $0.1 < p < 0.2$ with 4 d.f.
For the negative binomial, chi-square	= 4.463; $0.1 < p < 0.2$ with 2 d.f.
For Skellam's distribution, chi-square	= 1.537; $0.5 < p < 0.7$ with 2 d.f.

Discussion

Extended discussion at this point would be superfluous as there is little to choose between the distributions here.

It is difficult to assess the effect of selective application of contraception on the frequencies. The ideal sample for the present purpose would be one in which contraception was either inefficient or not extensively practiced. However, so far as I am aware, no appropriate recent data have been published on such a sample. *Faut de mieux*, one has recourse to data gathered at a time when contraceptive propaganda was impassioned, illegal (in the U.S. Mail) and relatively inefficient (Stopes, 1927), in contrast to the bland product of the 1930's (McCarthy, 1954). The trouble about such data is that (following the tradition of more delicate days), they fail to separate induced from spontaneous abortions.

I shall describe an attempt to fit one such set of data to double binomials, negative binomials and to Skellam's distribution.

Data collected prior to 1921 have been presented by Harris and Gunstad (1936). For a sample of foreign-born women resident in the U.S., they gave the frequencies with each possible combination of numbers of live births and spontaneous abortions. These women were ascertained by a visit to an obstetric hospital. Harris and Gunstad were aware of some of the consequences of their mode of ascertainment: If the outcome of the ascertainment-pregnancy is included, the incidence of abortion will be underestimated because abortions have a smaller chance of hospitalization than live births. Instead, Harris and Gunstad elected to ascertain by a live birth which they then ignored; none of their data has been contributed by women who aborted their every pregnancy, so it is clear that bias may remain. Nevertheless, the bias is probably not great.

TABLE 4. WOMEN IN THE HARRIS AND GUNSTAD SAMPLE WITH 2, 3, 4, 5, 6 AND 7 PREGNANCIES. FREQUENCIES OF WOMEN OBSERVED AND EXPECTED ON THE DOUBLE BINOMIAL, SKELLAM'S DISTRIBUTION AND THE NEGATIVE BINOMIAL

		Abortions								Total
		0	1	2	3	4	5	6	7	
2 Pregnancies	O	449	121	20						590
	D	441.9	119.5	28.6						590.0
3 Pregnancies	O	228	94	30	8					360
	D	243.5	78.5	30.9	7.2					360.0
	S	228.8	91.7	32.3	7.2					360.0
	N	225.3	100.2	27.3	5.9					358.7
4 Pregnancies	O	141	44	22	7	2				216
	D	134.1	47.9	22.3	9.9	1.8				216.0
	S	140.2	46.3	19.8	7.7	2.1				216.0
	N	136.9	53.0	17.8	5.7	1.8				215.2
5 Pregnancies	O	69	34	24	12	3	1			143
	D	82.4	32.0	15.2	9.4	3.5	0.5			143.0
	S	67.0	38.9	21.5	10.5	4.1	1.0			143.0
	N	63.8	44.3	21.3	8.7	3.2	1.1			142.4
6 Pregnancies	O	56	22	13	7	1	0	0		99
	D	53.3	22.6	10.1	7.6	4.1	1.2	0.2		99.0
	S	53.8	26.6	11.9	4.7	1.6	0.4	0.1		99.0
	N	52.7	28.6	11.5	4.1	1.4	0.4	0.1		98.9
7 Pregnancies	O	31	12	6	4	1	3	1	0	58
	D	29.3	13.6	5.6	4.5	3.2	1.4	0.4	0.0	58.0
	S	31.5	10.9	6.3	4.0	2.6	1.6	0.8	0.3	58.0
	N	29.1	13.9	7.1	3.7	2.0	1.0	0.6	0.3	57.6

O = observed.

D = double binomial.

S = Skellam's distribution.

N = negative binomial.

The fits to the three theoretical distributions are shown in table 4. In Harris and Gunstad's data, abortion rates did not vary much with gravidity at the time of ascertainment. Hence, there seemed no advantage in estimating ν_1/ν_2 separately for each gravidity group. So weighted averages of q_1 and q_2 were first computed, and then an over-all estimate of ν_1/ν_2 derived therefrom.

The results were:

For the double binomials chi-square = 21.131; $0.2 > p > 0.1$ with 15 d.f.

For the negative binomials chi-square = 11.58; $0.05 > p > 0.02$ with 5 d.f.

For Skellam's distribution chi-square = 3.054; $0.7 > p > 0.5$ with 5 d.f.

Again, the best fit is yielded by Skellam's distribution, but the present results can scarcely be said to clinch the matter.

A Further Test

The apparent superiority of the Skellam hypothesis suggests that an attempt to fit Whitehouse's (1930) data to Skellam's distribution might be attended with success. Table 5 shows the results of such an attempt.

Chi-square = 11.574, $p < 0.01$ with 3 d.f.

I have already shown that these data can be reasonably fitted by double binomials (James, 1961) so the poor fit of Skellam's distribution might plausibly be regarded as crucial, if one were satisfied with the data. However, they confound induced and spontaneous abortions.

TABLE 5. WOMEN IN THE WHITEHOUSE SAMPLE WITH 4, 5 AND 6 PREGNANCIES. FREQUENCIES OF WOMEN OBSERVED AND EXPECTED ON SKELLAM'S DISTRIBUTION

		Abortions							Total
		0	1	2	3	4	5	6	
4 Pregnancies	O	81	39	6	7	3			136
	E	84.6	29.2	13.9	6.3	2.0			136.0
5 Pregnancies	O	42	31	16	3	1	2		95
	E	44.4	27.2	14.3	6.4	2.2	0.4		95.0
6 Pregnancies	O	27	15	5	6	2	2	0	57
	E	27.4	13.1	7.7	4.6	2.6	1.2	0.4	57.0

O = observed.

E = expected.

Conclusion

Skellam's distribution makes a good fit to the data of Harris and Gunstad and of the Institute for Sex Research but a poor fit to Whitehouse's data. The double binomial makes a fair (but unimpressive) fit to all three. It is possible that the anomalous nature of these results is attributable to characteristics of the data which render them questionably suitable for the present purpose; viz, the older data fail to discriminate between induced and spontaneous abortions, while more recent data show evidence of selective applications of contraception which violates the assumptions underlying the theoretical distributions.

ABORTION, MATERNAL AGE AND BIRTH ORDER

It has been commonly observed that spontaneous abortion rates correlate with birth order (Hudson, and Rucker, 1945; Javert, 1957; Rucker, 1952; Schoeneck, 1953; Stevenson, and Warnock, 1958; Shapiro, Jones, and Densen, 1962) and with maternal age (Gebhard and *et al.* 1958; Javert, 1957; Rucker, 1952; Stevenson and Warnock, 1958; Tietze, Guttmacher and Rubin, 1950). To the knowledge of this writer, however, only one attempt has been made to sort out the relative influence of these two variables (Warburton, 1961). However, in view of the empirical concurrence of Shapiro *et al.* (1962) with Erhardt and Jacobziner (1956) that the relationships are not linear, it would seem that Warburton's conclusions from a linear regression analysis may be misleading.

Be that as it may, it is usual to infer from the well-documented correlations that a woman's risk of aborting a pregnancy increases both with her age and with birth order (Javert, 1957). I shall call this the "causal" hypothesis. Its advocates might invoke, for instance, explanations involving:

- (1) gradually deteriorating features of the intrauterine environment, or
- (2) degradation of the ovum in the interval between the differentiation of the primary oocytes (possibly before puberty) and ovulation, or
- (3) cumulative susceptibility of the maternal gonads to lethal mutagenic agents.

Of particular interest in this connection is the association of some forms of chromosomal anomalies with maternal age. Chromosomal anomalies have been shown to be associated with some spontaneous abortions (Penrose and Delhanty, 1961; Delhanty, Ellis and Rowley, 1961; Schmid, 1962) but the types of anomaly involved (triploidy and reciprocal translocations) have not, to my knowledge, been linked with maternal age.

It might be observed that nondisjunction of chromosome number 21 presumably gives rise to an equal number of monosomics as it gives to trisomic mongols, the monosomics dying, however, in utero. Such a mechanism might be expected to be intimately linked with maternal age; but since it would occur in about one pregnancy in 600 (the incidence of mongolism), it could be expected only to account for perhaps one abortion in 100.

During the remainder of this report this type of "causal" hypothesis will be questioned. Instead, data will be presented to support what will be called the 'artifact' hypothesis, viz that:

- (1) Some women (here called "abortion-prone") are more likely to abort their pregnancies than other "abortion-resistant" women.
- (2) Abortion-prone women have more pregnancies on the average than abortion-resistant women.
- (3) Abortion-prone women have pregnancies at higher ages on the average, and
- (4) The combination of these facts is mainly responsible for the observed correlations of abortion rate with maternal age and with gravidity.

It will be argued that the high spontaneous abortion rates for advanced age and gravidity categories are due to the higher proportions of abortion-prone women in these categories.

The Sample

The data used in the present analysis were elicited partially from the ISR Sample. Other data were provided by 822 additional women who were also interviewed by I.S.R. staff. These women also were white and had not been to prison, but they differed from the ISR Sample in that they: (a) were 30 plus years of age at the time of their marriage, or (b) were 36 minus years at the time of interview (or both).

I shall call these 822 women the ISR Sample 2.

In women in the ISR Sample the percentages of pregnancies aborting in the first five birth orders were 15.6, 13.7, 20.4, 20.0, and 29.6. For subsequent birth orders, the over-all percentage was 25.4. In the ISR Sample the ages at pregnancy were coded in 5-year intervals (20 and less = 1; 21-25 = 2,36 and over = 5). The percentages of pregnancies aborting during these five coded maternal ages were, respectively, 15.2, 20.2, 20.6, 22.0, and 27.5. Each of these percentages is based on a total of more than 70 pregnancies, so that the over-all rise in each case is clearly not attributable to chance.

Method: Analysis

The inference of a maternal age effect in spontaneous abortion has hitherto been drawn from the correlation of spontaneous abortion rates with birth order

and with maternal age (correlations which are exemplified, as just noted, in the present data). However, these correlations could exist even if the causal hypothesis were false. This possibility may be tested by analyzing the data in a manner suggested by Slater (1962). His description of his method may be quoted:

"When an individual comes m th in order of a sibship of n individuals —i.e., in the total of children borne by his mother—his ordinal position may conveniently be designated by the figure $(m-1)/(n-1)$. This expression varies in value between the limits of 0 and 1, with a mean value which in a random collection of individuals tends towards 0.5. Within sibships of a stated size the values are not normally distributed, since each ordinal position is equally probable; but the means of values obtained on a series of individuals will tend to be normally distributed. The variance of values of this expression is easily calculated, so that the probability of a deviation from the expected mean of 0.5 can be reliably estimated. Not only the expected mean but also the expected variance of a series of observations may be calculated; for it is readily shown that the average theoretical contribution of each single observation to the total variance is $(n+1)/12(n-1)$ —an expression which takes high values in small sibships and approximates to $1/12$ as the sibship size increases."

In contrast to the Greenwood-Yule Method (Greenwood and Yule, 1914), Slater's analysis offers a means of detecting heterogeneity.

Findings: Birth Order

Tables 6 and 7 show the distribution of birth order of spontaneous abortions in each of the two samples; table 8 shows how the mean birth order, the

TABLE 6. ORDER OF BIRTH OF 241 SPONTANEOUS ABORTIONS
IN THE ISR SAMPLE

Sibship Size	Order in Sibship									
	1	2	3	4	5	6	7	8	9	10
1	19									
2	11	13								
3	18	9	15							
4	22	16	18	12						
5	11	7	6	8	11					
6	4	5	5	4	4	3				
7	2		1	2	3	2	4			
8					1			1		
9										
10	1								1	1
11		1								

TABLE 7. ORDER OF BIRTH OF 180 SPONTANEOUS ABORTIONS
IN THE ISR SAMPLE 2

Sibship	Order in Sibship								
	1	2	3	4	5	6	7	8	9
1	40								
2	20	23							
3	19	14	11						
4	6	6	7	8					
5	1	4	2	2	3				
6	1	1	1	1	1				
7	2	1	1	1	1	1	1		
8									
9	1								

variance and the standard error of the mean have been calculated for the pooled sample. The pooling is felt to be justified by the similarity of the two samples. Of course, by including a group of women with short reproductive lives (the ISR Sample 2) any age-effect would be lessened. But it may be verified that the relevant parameters of the two distributions are very similar. The principal conclusion is that the mean birth order of the abortions was 0.4846 with a standard error of 0.02157. These values are compatible with the hypothesis that birth order *per se* has no influence on the probability that a pregnancy will abort. It is true that the total variance observed, 60.81, is slightly higher than the expected value of 57.82, so the possibility of heterogeneity is not ruled out, although the limited extent of this difference would seem to rule out any marked age-effect in the majority of cases.

In interpreting these results, one makes the assumption (as one does with the Greenwood-Yule method) that fertility is not affected by an affected birth. If fertility were increased after such a birth, the analysis would yield the spurious suggestion that abortion risks are higher in the lower birth orders. It has been shown that this assumption is violated—a woman is more likely to engage in a further pregnancy after an affected birth. However, this excess fertility diminishes with birth order; to demonstrate this, data were taken on those ISR women who had aborted an arbitrarily chosen value of 30 per cent or more of their pregnancies. They will be called the “abortion-prone” subsample. Within this subsample the incidence of spontaneous abortion among *terminal* (*i.e.*, for each woman, the last pregnancy in her history) pregnancies was compared with the corresponding figure for their other pregnancies. For this purpose, women with only one pregnancy were omitted, and in those cases where the history ended with an induced abortion, the terminal pregnancy is taken as the last one which is not artificially terminated. The incidence of spontaneous abortion among the terminal pregnancies of abortion-prone women was 50.7 per cent and the incidence of spontaneous abortion among other pregnancies of abortion-prone women was 53.5 per cent. These incidences seem sufficiently close to warrant the conclusion that an abortion is followed only by additional fertility if it occurs relatively early in the sibship (otherwise these terminal pregnancies would show a less marked tendency to abort). This being so, the spurious negative birth-order effect would not be expected to be very great in the present case. It follows that if there really is a positive birth-order effect (*i.e.*, if the “causal” hypothesis is true), then it must have been very slight to have been swamped by a statistical artifact which it has been argued, must be small.

So it seems to follow that there is little evidence for the causal hypothesis; *per se* neither birth order nor, by inference, maternal age affect the probability that a pregnancy will abort.

Maternal Age

It is usual, in identifying a maternal age-effect, to contrast the frequency distribution of maternal ages of affected births with a comparable distribution for normal births. The maternal age distribution for spontaneous abortions and for all live births in the ISR Sample are contrasted in Fig. 1.

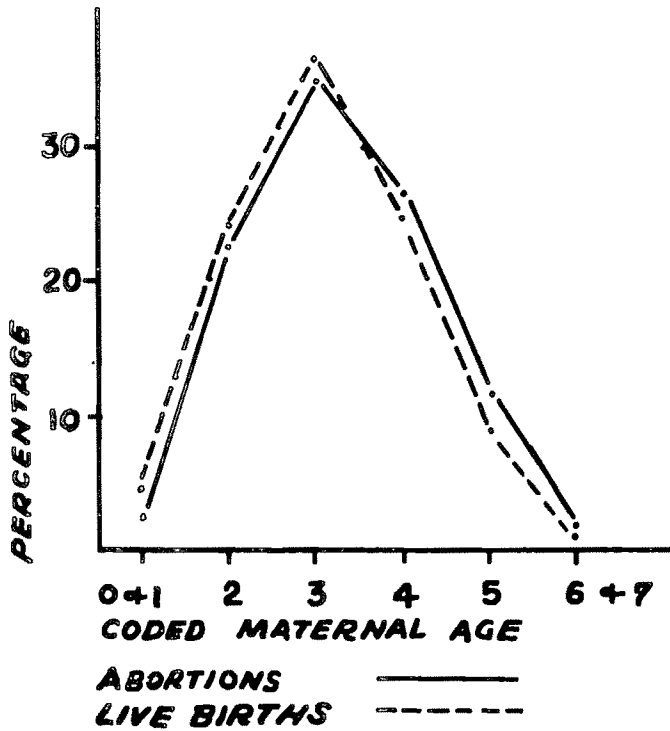


FIG. 1. Pregnancies in the ISR Sample. Percentages of total abortions and of total live births occurring at the given coded maternal ages.

This point will be treated cursorily only for two reasons: (a) It is debatable whether any available maternal age distribution is strictly comparable with the distribution for abortions here presented because it is known that the ISR Sample is not a representative sample of the American population (Gebhard *et al.*, 1958, pp. 18-23); (b) in any case, this form of analysis does not discriminate between the "causal" and the "artifact" hypotheses.

For these reasons the question of maternal age will not be further treated. This perhaps is not too serious, for as Slater (1962) says: "Even when we are primarily interested in maternal age, we may find that birth order supplies more reliable information...it may not be safe to compare maternal age for groups derived from different regions, different social classes, or different epochs. The subject's own sibship provides the control data which can be relied on to equalize such adventitious differences, if the datum taken is birth order."

A Further Test

To put the two hypotheses to a test, it was conjectured that if the causal hypothesis were true, then the correlations of abortion rates with maternal age and with gravidity should appear in at least one of the subsamples, the abortion-prone and the abortion-resistant subsamples. To check this, spontaneous abortion rates were computed by 5-year age intervals within both of the ISR subsamples. Secondly, spontaneous abortion rates were computed by gravidity

rank within each of the subsamples (for this purpose, induced abortions were acknowledged in the following sense: for a woman whose history comprised an induced abortion for her first pregnancy and a spontaneous abortion for her second, only one entry is recorded—a spontaneous abortion for gravidity rank 2).

In the subsamples, the results for the lowest two maternal age categories were pooled. Then the percentages of pregnancies aborting in advancing age categories in the abortion-prone subsample were 58.4, 52.7, 56.2 and 53.6. The corresponding figures for the abortion-resistant subsample were 2.2, 3.5, 2.6 and 2.9. The percentages of pregnancies aborting in the first four birth orders in the abortion-prone subsample were 60.8, 55.0, 57.3 and 46.4. For subsequent birth orders, the over all percentage was 53.0. The corresponding figures for the abortion-resistant subsample were 2.3, 0.3, 2.9, 4.3 and 5.3. The percentages italicized are for totals of between 50 and 100; all other totals exceed 100. One would conclude that spontaneous abortion rates remain largely unaffected by age. The figures for birth order are not so conclusive. In abortion-prone women there seems no evidence for a birth order effect, but in abortion-resistant women there is a slight rise in incidence from the second birth rank onwards. However, even if this rise is not due to chance, it is associated only with a small minority of abortions.

It is concluded that the lack of association of spontaneous abortion rates with age or with gravidity within either of these subsamples strongly militates against the theory that there is a direct causal connection between age and/or gravidity and the great majority of spontaneous abortions.

Assuming then, that the "causal" hypothesis is not true, it is interesting to examine the basis of the correlations which, confusingly, lend it credence. For this purpose, the mean number of pregnancies in the abortion-prone and abortion-resistant subsamples will be contrasted. It should be noted at the outset that the following considerations merely attempt to explain the origin of the correlations which make the "causal" hypothesis plausible; they are in no way

TABLE 8. CALCULATION OF MEAN BIRTH ORDER OF 362 SPONTANEOUS ABORTIONS FROM SIBSHIPS OF 2 OR MORE IN THE POOLED SAMPLE AND OF STANDARD ERROR OF MEAN

Sibship Size	No.	Sum of Values	Sum of Squares
2	67	36.0000	36.0000
3	86	37.5000	31.7500
4	95	44.0000	33.5556
5	55	28.2500	22.3125
6	30	13.6000	9.2000
7	22	12.5000	9.8611
8	2	1.5714	1.3265
9	1	0.0000	0.0000
10	3	1.8889	1.7901
11	1	0.1000	0.0100
Totals	362	175.4103	145.8058
Mean		0.4846	
Correction term			84.9966
Total variance			60.8092
S. E. of mean		0.02157	

intended to add directly to the evidence against the "causal" hypothesis. For if the "causal" hypothesis were true, then women ascertained by aborting would, on the average, be older at pregnancy and have had more pregnancies anyway. For this observation I am indebted to Professor L. S. Penrose.

Mean Number of Pregnancies of Abortion-Prone and Abortion-Resistant Women

In the search for an explanation of the apparently unabated incidence of several disorders against which there is known to be genetic selection, it has been noted that parents are inclined to compensate for a fetal or infantile death by initiating a further pregnancy. This has been reported, for instance, in microcythemia (Silvestroni, Bianco, Montalenti and Siniscalco, 1950), acholuric jaundice (Race, 1942), and erythroblastosis fetalis (Glass, 1950). Such a tendency to compensate for fetal loss has also been reported among women with a high incidence of spontaneous abortion (Winkelstein, Stenchever and Lilienfeld, 1958). However, since these abortion-prone women had been ascertained by having survived one or more myocardial infarctions, the point needs clarification.

Using a *t* test, it was found that the abortion-prone subsample had had a higher mean number of pregnancies (2.79) than the abortion-resistant subsample (2.42). ($t = 3.07$, $p < 0.01$.)

The causes of these additional pregnancies among the abortion-prone women are not germane to this report; however, since it seems that they may be readily identified, I shall try to identify them. I have no data on the contraceptive practice of the ISR Sample; however, data provided by Reed and Kelly (1957) on a comparable group of women were examined. For each woman, the third pregnancy was classified by whether it was live or aborted, and the subsequent pregnancy (*i.e.*, the fourth) was classified by whether it was preceded by contraceptive efforts or not. Pregnancies between which an intervening induced abortion had occurred were not counted, and stillbirths were counted as spontaneous abortions. Table 9 shows the frequencies (in Reed and Kelly's sample) of contraceptive practice prior to the fourth pregnancy, by the outcome of the

TABLE 9. PREGNANCIES OF WOMEN IN REED AND KELLY'S
(1957) SAMPLE

Outcome of preceding (index) pregnancy	Frequencies of Planned vs. Unplanned Pregnancies of Gravidity Rank + by the Outcome of the Immediately Preceding Pregnancy	
	Subsequent Pregnancy (Unplanned despite contraception)	(No planned contraception)
Abortion or stillbirth	1	18
Live birth	10	23

For this partition and the more extreme one, the sum of Fisher's exact probabilities is $p = 0.032$.

index (third) pregnancy. The sum of Fisher's exact probabilities for this partition and the more extreme one is $p = 0.032$. It may be verified that similar extreme partitions are yielded if the first or second pregnancies are chosen as index. It may be inferred then that spontaneous abortions are more

likely than live births to be followed by planned pregnancies (*i.e.*, pregnancies occurring in the absence of contraceptive efforts).

Fisher (1930) had suggested that families tend to fill up to a certain average size for a given population or stratum within the population, and the point has received confirmation from, *inter alia*, the Indianapolis Study (Kiser and Whelpton, 1958). It seems that for many people the ideal family size is two children; it is suggested therefore that abortion-prone women repeatedly undertake pregnancies in order to achieve this ideal; it simply takes more pregnancies in their case.

Mean Age At Pregnancy of Abortion-Prone and Abortion-Resistant Women

It has been argued that the correlation of spontaneous abortion rates with gravidity is due to the fact that abortion-prone women have more pregnancies on the average. It may also be shown that abortion-prone women have pregnancies at more advanced ages on the average. In the ISR Sample, the ages at pregnancy were coded in 5-year intervals (16-20 = 1; 21-25 = 2....41-45 = 6). The mean coded age at pregnancy of abortion-prone women was 3.29, while the corresponding figure for abortion-resistant women was 3.09. For the difference, $t = 4.02$ and $p < 0.01$. It follows that within maternal age categories, the proportion of gravidae who are abortion-prone correlates with maternal age.

SUMMARY AND CONCLUSIONS

1. Abortion-probability varies from woman to woman but remains relatively constant within a given woman.
2. Abortion-prone women have more pregnancies, on the average, than other women.
3. Abortion-prone women have their pregnancies at higher ages, on the average, than other women.
4. These facts (rather than a direct causal nexus) account, partially at least, for the correlation of spontaneous abortion rates with maternal age and with gravidity.
5. The outcomes of adjacent pregnancies show no evidence of positive correlation with one another.
6. Selective application of contraceptive techniques after families have reached the desired number of live births, interferes with the randomness assumed by interpretations based on curve-fitting procedures.
7. The data here examined seem inadequate to discriminate between the hypotheses that abortion-probabilities are: a) normally distributed, and b) bimodally distributed.
8. This question is unlikely to be settled (using the present methods anyway) until data are available which separate induced from spontaneous abortions in a population which does not use contraceptive methods.

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nucleotide (TPN); 0.6 mM sodium G-6-P; and hemolysate, in a total volume of 1.0 ml. The method of estimating the dilution of each hemolysate and adjusting activity of G-6-PD to units per 100 ml. of erythrocytes has been outlined (Kirkman and Riley, 1961). A unit of G-6-PD was defined as the amount of enzyme necessary for reduction of 1 μ M of TPN per minute in the described assay solution at 25°C. In a cuvette of 1 cm. light path this represented an optical density change of 6.2 per minute at 340 m μ wave length. Hemolysates showing less than 100 units per 100 ml. of erythrocytes (less than 60 per cent of the average adult value) were assayed also with sodium-6-phosphogluconate and sodium G-6-P by the principle of Glock and McLean (Kirkman and Riley, 1961). Activity less than 50 units per 100 ml. was considered indicative of primaquine sensitivity in males.

Preparations of Hemolysates for Electrophoresis

Each supernatant hemolysate was adjusted to 0.05 M Tris (HCl), pH 8.0; 2.7 mM sodium ethylenediaminetetraacetate (EDTA, pH 7); 7mM β -mercaptoethanol; and 0.013 units G-6-PD per ml. This final dilution of hemolysate provided concentrations of hemoglobin and G-6-PD comparable to a 100-fold to 160-fold dilution of red cells. Hemolysates from primaquine-sensitive persons were diluted to a final concentration of 0.4 Gm. hemoglobin per 100 ml. regardless of activity. Each hemolysate was inspected for faint turbidity; if present, it was removed by centrifugation of the hemolysate at 16,000 G for 20 minutes. A difficulty occurred with hemolysates that were allowed to stand with a lower ionic strength, or higher concentration of hemoglobin, than specified here. This was signaled by appearance of a fine turbidity that could not be cleared by subsequent alteration of concentration or ionic strength of the hemolysate. Unless this turbidity was removed, the G-6-PD and hemoglobin of the sample left the slot both prematurely and with slurring. A person outside of this laboratory received the final hemolysates, returned them in coded test tubes, and mailed the code to another outside person.

Electrophoresis in Starch Gel

The starch gel was prepared from a mixture of: 50 ml. of 0.5 M Tris (HCl), pH 8.8; 5 ml. of 0.27 M sodium EDTA, pH 7.0; 67.5 Gm. of hydrolyzed starch; and 450 ml. of water (Kirkman, 196 lb.). The mixture was heated and poured, as described by Smithies (1959), into an 8-channel vertical electrophoresis tray. TPN, 5 ml. of 1 mM, was mixed thoroughly into the molten gel just before the gel was poured. After the tray remained at room temperature for 2 hours, the slot mold was removed, and a single strip of Parafilm was placed over the empty slots. White petroleum jelly (60°C) was poured over the gel and smoothed with a spatula to a thickness of approximately 3 mm. The tray was placed in a 2°C room for 5 hours. Removal of the strip of Parafilm exposed the sample slots and narrow adjacent areas of gel. Strips of filter paper were carefully placed in the slots for 20 to 30 seconds, then removed. Overlying buffer was blotted from the adjacent gel. Each slot received 0.05 ml. of sample. The slots and exposed gel were covered with 3 to 5 mm. of molten (50°C) petroleum jelly.

Reservoir solution consisted of 0.05 M Tris (HCl), pH 8.8; 0.05 M sodium chloride; and 2.7 mM sodium EDTA, pH 7.0. The cathodic reservoir compartment nearest the gel contained 10 μ M TPN. Electrophoresis was performed at 2°C for 16 hours at a gradient of 4 volts per cm.

Stain for G-6-PD

The gel was sliced and labeled on its anodic end with 0.01 M reduced diphosphopyridine nucleotide (DPNH). A mixture of 5.0 ml. of 0.5 M Tris (HCl), pH 8.0; 0.5 ml. of 0.5 M MgCl_2 ; 3.5 Gm. of starch; and 30.0 ml. of water; was heated until molten, then cooled to 45°C. The mixture then received 5 ml. of 0.17 M potassium cyanide, 1.0 ml. of 20 mM G-6-P, 0.25 ml. of 10 mM TPN, 7.5 ml. of 30 mg. per 100 ml. nitro BT, and 0.45 ml. of 1.1 mM (0.5 per cent) methylene blue. (MgCl_2 and potassium cyanide can be omitted in studies of G-6-PD from human erythrocytes. Also, concentration of TPN in the gel slab can be reduced when stable G-6-PD is used.) The mixture was cooled to 40°C and poured over the sliced gel. The gel was placed in the dark at 2°C for 20 minutes, then incubated in a humidified container in the dark at 30°C for 3 hours. After emersion of the gel in tap water, overlying developing mixture could be removed, and the pattern on the sliced gel could be observed. Gels were stored under water at 2°C. Methylene blue and hemoglobin diffused from the gel during storage, leaving the purple bands of reduced tetrazolium against a background of white gel. Two observers independently recorded their interpretations of the gels and mailed their readings to the person who received the code.

Other Procedures

Erythrocytic G-6-PD was purified 100-fold (Kirkman *et al.*, 1960) to 20,000-fold (Kirkman, 1962b) for electrophoretic observations of minor bands of the enzyme. These preparations were dialyzed for 16 hours against a solution of 0.05 M Tris, pH 8.0; 2.7 mM EDTA; 7 mM β -mercaptoethanol; and 10 μ M TPN. They were adjusted with dialysis solution to 0.5 units of G-6-PD per ml. before placement in starch gel.

RESULTS

Three electrophoretic patterns of G-6-PD were found in erythrocytes of Negroes (Fig. 1): A slow G-6-PD, identical in migration to that encountered in Caucasians, a slightly faster G-6-PD, and a broad band that seemed to represent a mixture of both the slow and fast enzyme. These will be designated B, A, and AB, according to the nomenclature of Boyer *et al.* (1962). A mixture of the first two types of hemolysates provided a band identical to AB bands. The proximity of the B and A bands suggested that occasional misreadings might occur and necessitated the blind readings in order that biased interpretation or reinterpretation might be averted. Nevertheless, readings of the two observers were concordant on 289 of the 291 samples in the blind study. Two samples, both from mothers of B sons, were interpreted differently by the observers: G-6-PD of the first mother was called both B and AB, that from the second

mother, both A and AB. The two observers recorded uncertainty in their readings of one of these samples. In addition, another woman was thought to have a son with both B and A G-6-PD: Both observers recorded his band as an obvious mixed band and his mother's enzyme as B. He and his mother were recalled and

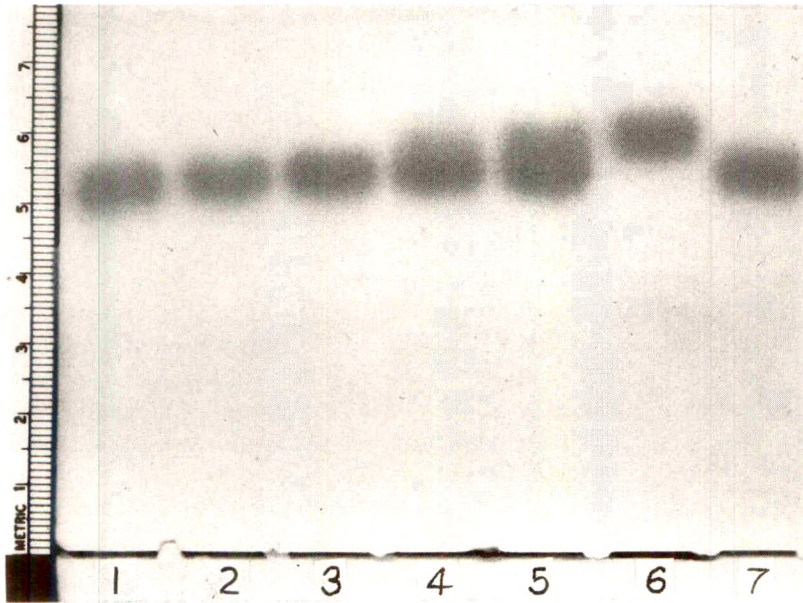


FIG. 1. Migration of glucose-6-phosphate dehydrogenase on vertical starch-gel electrophoresis. The gel, stained for activity, contained the following types: channels 1-3 and 7, B; channels 4 and 5, AB; channel 6, A.

again tested under blind conditions. On the second test, the mother was thought to have an obvious AB band, and the son a B band. The initial AB reading on this son probably arose from a tube mix-up, occurring during the approximately one thousand tube transfers that were required for the study. These three mothers and their sons were excluded from all tables. Of the remaining 135 mothers, 119 were women whose first-tested son had normal activity of G-6-PD. The phenotypes of these women and their first-tested sons, all determined under blind conditions, appear in the top three rows of table 1.

TABLE 1. ELECTROPHORETIC PHENOTYPES OF G-6-PD FROM RED CELLS OF NEGRO MOTHERS AND THEIR FIRST-TESTED SONS

		Mothers				Totals
		B	AB	A	A ⁻	
Sons	B	71	17	0	0	88
	AB	0	0	0	0	0
	A	0	25	6	0	31
	A ⁻	8	6	1	1	16
	Totals	79	48	7	1	135
Supplementary group						
	A ⁻	3	1	2	1	

Sixteen of the 135 women had first-tested sons with deficiencies in activity of G-6-PD (primaquine sensitivity). Their electrophoretic patterns are given in table 1, fourth row. Of 19 primaquine sensitive Negro males encountered in this series and 29 encountered outside of the series, all had an erythrocytic G-6-PD which migrated at the same rate as the fast G-6-PD of Negro males with normal activity of the enzyme. Boyer and Porter (1962) have made similar observations. Like the A band, the enzyme from these sensitive subjects migrated so close to the B band that the difference would not have been noticed with isolated observations by earlier methods (Marks, Szeinberg and Banks, 1961; Kirkman, 1961b). This slightly faster migration was also seen with 16,000-fold purified sensitive G-6-PD.

When compared by electrophoretic mobility the sensitive and A enzymes were indistinguishable; but the quantitative assay for G-6-PD permitted a separate designation for the phenotype of sensitive Negro males (A^- by the nomenclature of Boyer *et al.*, 1962). The same designation, A^- , was given to two mothers with enzymic activities less than 50 units per 100 ml., as these had only a fast band. The hemolysates from sensitive subjects could be identified readily on starch gel by their faint, fast enzymic band and relatively intense hemoglobin band. This identification precluded their incorporation into a blind study.

No attempt was made to designate women as heterozygous for primaquine sensitivity on the basis of enzymic assay or on the basis of a faint A band mixed with a strong B band. Of the 6 AB mothers of sensitive sons in table 1, however, five had an A band less intense in color than their B, while eight of 42 AB mothers of normal sons had an A less intense than their B band (table 2). This

TABLE 2. RELATIVE INTENSITIES OF A AND B BANDS IN AB FEMALES.
(-3, equivocal A; -2, faint A; -1, moderately reduced A; 0,
A and B equal; +1, moderately reduced B, +2, faint B)

		-3	-2	-1	0	+1	+2
				Mothers			
	B	0	1	2	10	4	0
Sons	A	0	1	4	15	4	1
	A^-	2 [†]	2	2	0	1	0
	A^-*	0	1	0	0	0	0
		0	2	Girls	6	3	0
				2			

*Supplementary.

†Considered as one B and one AB in table 1.

difference is significant on a 2 x 2 contingency table at $P < 0.005$, when corrections are applied for small numbers (Snedecor, 1956). Yet some of the AB mothers with faint A bands had A sons. The findings with mothers of sensitive sons were supplemented (table 1, bottom row) by continuing the survey, without electrophoretic typing of normal sons and their mothers, until an additional seven unrelated, sensitive sons were found.

Triple allelism with simple co-dominance would preclude the occurrence of A^- sons from B mothers, unless faint fast bands (representing the A^-

enzyme) were sometimes missed in mothers who are heterozygous for primaquine sensitivity. Fig. 2 offers evidence that this may occur. B and A⁻ hemolysates were mixed in proportions that might simulate hemolysates of women

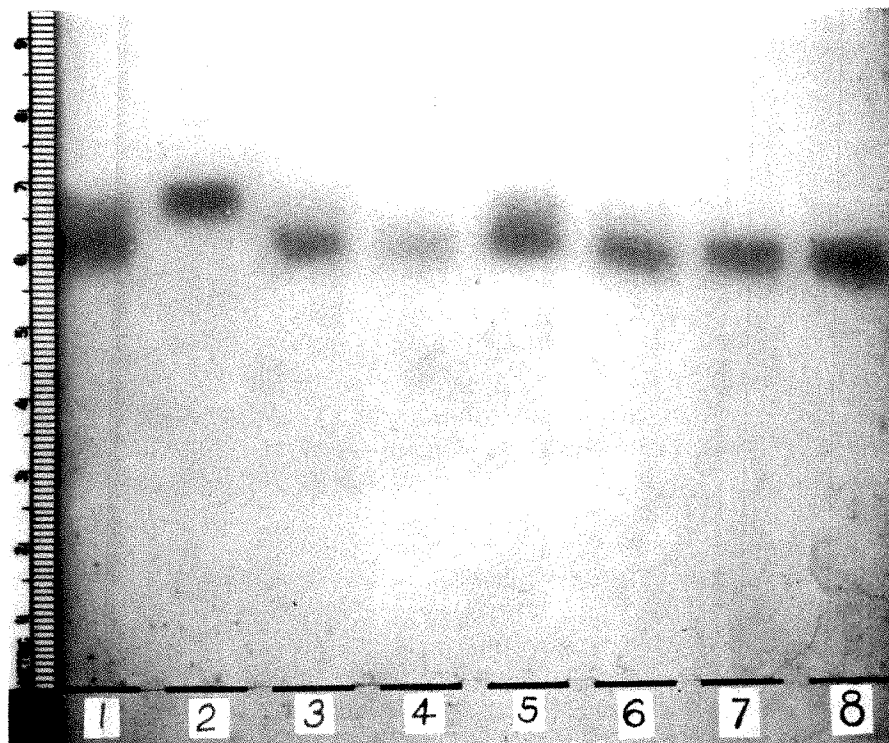


FIG. 2. Relative intensity of staining of glucose-6-phosphate dehydrogenase after vertical starch-gel electrophoresis of mixtures of a normal and a primaquine-sensitive hemolysate. The samples contained normal: sensitive hemolysate in the following proportions (as percentage of total hemoglobin): channels 1 and 5, 25: 75; channels 3 and 6, 40:60; channels 4 and 8, 60:40. Samples of channels 1, 3, and 4 each contained 0.007 units of total enzyme per ml.; channels 5, 6, and 8 each contained 0.01 units of total enzyme per ml. Channel 2 contained only sensitive hemolysate (0.005 units per ml.); channel 7, only normal hemolysate (0.01 units per ml.).

heterozygous for B and A⁻. At proportions less than 60:40 (hemoglobin of A⁻: hemoglobin of B) or 10 units of A⁻: 60 units of B, the A⁻ bands became imperceptible. Observations of electrophoretic patterns in sickleemia (Wells and Itano, 1951) and of enzymic activity in primaquine sensitivity, galactosemia (Donnell *et al.*, 1960), and acatalasia (Takahara *et al.*, 1960), offer precedence that the phenotype of heterozygous subjects resembles the phenotype that would be expected of an approximately equal mixture of blood from homozygous or hemizygous persons.

The 39 Negro girls had the following phenotypes: 23 B, 13 AB, and 3 A. Some of the mothers had more than one son tested. Patterns of the additional sons are listed in table 3.

TABLE 3. ELECTROPHORETIC PHENOTYPES OF G-6-PD FROM RED CELLS OF NEGRO MOTHERS AND THEIR SONS

Mother	First-Tested Sons	Other Son(s)	No. of Such Mothers
B	B	B	5
AB	B	A	1
AB	A	B	1
AB	A	A	1
B	B	A ⁻	2
B*	A ⁻	B and A ⁻	1
A	A ⁻	A§	1
B†	A ⁻	A ⁻	1

*The only mother with more than 2 sons tested.

†From supplementary group.

§Erroneously listed as first-tested son in an earlier report (Kirkman, 1962a).

DISCUSSION

Previous studies have shown that the deficiency in activity of human G-6-PD is sex-linked. The findings to date (Boyer *et al.*, 1962; Kirkman, 1962a) suggest that the electrophoretic difference also is sex-linked. Yet several circumstances point to a need for cautious and formal evaluation of the mode of inheritance: (1) the complex nature of human G-6-PD, as indicated by its ability to exist in a number of metastable states and to undergo reversible alterations in characteristics after relatively mild environmental changes; (2) the lack of precedents from which to draw intuitive experience with sex-linked control of protein structure; (3) the initial misappraisal of the mode of inheritance of the electrophoretic difference; (4) the closeness with which the data will fit some autosomal and alternative models (appendix, table 6). Moreover, the expense of substrates used in these studies prompted an attempt to extract information from the data as efficiently and completely as possible.

An appendix provides descriptions of various models and the methods for testing them. It is necessary to consider at this point only certain conclusions drawn from these computations, as they relate to requirements for further studies. Of the autosomal models that occurred to us, all failed significantly to fit the data. Two types of sex-linked models are compatible with the data: one, model 6 in appendix, involves two alleles at each of two loci; another, model 7, involves three alleles at one locus. Owing to apparent exceptions to sex-linked transmission reported by Boyer *et al.* and to our own inability to demonstrate A⁻ bands consistently in A-B females, at least a several-fold increase in number of subjects would seem necessary to distinguish between the two models. The required number of subjects might be reduced by electrophoresis, as described here, of both hemolysates and lysates of leukocytes from each female subject. Heterozygosity for A⁻ would be suggested by observations of a B band coupled with a dark fast band from leukocytes and a faint or absent fast band from hemolysate. Table 2 reveals, however, that some misclassifications could arise even from this method.

The observations of Boyer *et al.* (1962) also suggest sex-linked inheritance of the electrophoretic difference, but differ from our observations in several

details. They find: (a) the G-6-PD partially purified from red cells of males migrates as two bands; (b) hemolysates of deficient subjects often show no band; (c) partially purified G-6-PD from red cells of sensitive Negro males migrates atypically or as B; (d) hemolysates of females can not be classified accurately for presence of A and B bands. In our hands: (a) nearly all of the activity of males migrates as a single band; (b) hemolysates of deficient subjects always show a faint band; (c) both 60-fold and 16,000-fold purified enzymes from red cells of sensitive Negro males migrate as fast bands; (d) hemolysates from females can be classified accurately for presence of A and B bands (but not for A⁻ bands). These differences seem to arise from differences in technique. The observations of Boyer *et al.* are drawn from electrophoresis in a discontinuous buffer system without added TPN or EDTA; the observations reported here are drawn from electrophoresis in the presence of TPN and EDTA but without a discontinuous buffer system. We find that electrophoresis in the absence of both TPN and EDTA results in over 90 per cent loss of activity.

A relatively large variance has been reported for activity of erythrocytic G-6-PD from women who are heterozygous for primaquine sensitivity. Beutler, Yeh and Fairbanks (1962) cite this as evidence supporting mosaicism of X chromosomes in human females. The variation in relative intensity of fast and slow G-6-PD in heterozygous females also seems rather large, perhaps larger than the variation in relative quantities of hemoglobin S and A in sickle cell anemia, and sufficiently large to discourage those who feel that females heterozygous for primaquine sensitivity might always be detected from activity alone, if only a sufficiently accurate enzymic assay were available. Yet the existing variation might be explained by modifying effects of other genetic and environmental factors, rather than by mosaicism.

This study provided several additional findings. Fig. 3 shows a minor band of G-6-PD which appeared just behind the major band after large amounts of 100-fold purified G-6-PD were subjected to electrophoresis. Such preparations exhibited no band when 6-phosphogluconate replaced glucose-6-phosphate in the developing mixture. The minor and major band seemed proportionately reduced in intensity in preparations from primaquine-sensitive Negroes (Fig. 3). Both A⁻ and A males had a minor band that migrated slightly faster than the minor band of B persons. This difference in migration, however, was much less than that between major bands. A minor band was not seen in two preparations purified 15,000 to 20,000-fold but was faintly visible in one preparation purified 17,000-fold. The "holes" in the major bands are diffusion artifacts, apparently caused by depletion of substrate in the developing gel overlying very large amounts of activity.

The use of finger-prick blood proved to be less of a handicap than anticipated. No difficulty was encountered in washing such small amounts of red cells or in removing stroma. As the volume of red cells could not be directly measured conveniently, activity of G-6-PD was corrected for variations in dilutions by a measurement of optical absorbancy of hemoglobin directly in the cuvettes. The mean activity of G-6-PD from the 21 mothers of A⁻ sons, excluding two mothers who were called A⁻ (table 1), was 136 units per 100 ml. of red cells, with a

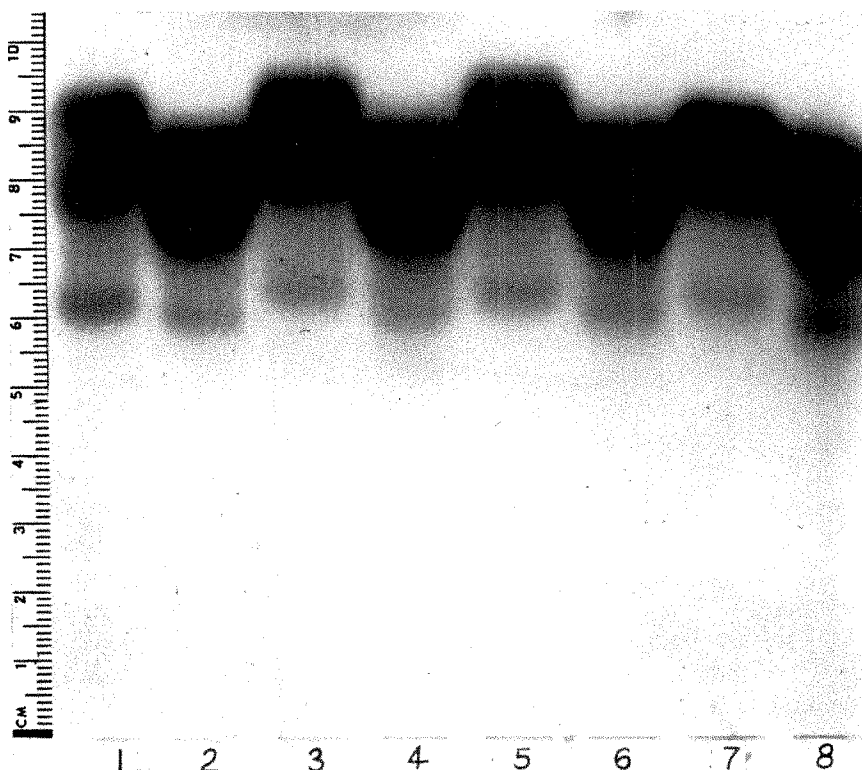


FIG. 3. Migration of minor bands of glucose-6-phosphate dehydrogenase on vertical starch-gel electrophoresis. A and B enzyme were purified 100-fold and diluted to 0.5 units per ml. Channels 1, 3, and 5 received A; channels 2, 4, 6, and 8 received B. Channel 7 received 170-fold purified A-enzyme (0.33 units per ml.).

standard deviation of 49. The 119 mothers whose first-tested sons were A or B had a mean activity of 214 with a standard deviation of 49. From the latter group an approximation was made of values for mothers who were homozygous-normal for primaquine sensitivity; 15 women whose activities matched those of table 1, row 4, were removed from the group. The remaining mothers had a mean activity of 223 with a standard deviation of 46. These standard deviations are slightly higher than those reported in studies with larger volumes of blood.

The relative intensities of hemoglobin and reduced tetrazolium (enzymic activity) on the sliced gel seemed to permit detection of primaquine-sensitive males. In gels which exhibited a boundary effect, the position of the hemoglobin facilitated the reading of the position of G-6-PD; for the leading edge of hemoglobin A fell immediately behind the trailing edge of B G-6-PD (Fig. 4). Hemoglobins A, C, and S were seen on the sliced, unstained gel, and hemoglobin A₂ could be seen after the unused half of the gel was stained with Amidoschwartz (Fig. 4). A 33-fold further dilution of the hemolysate provided a band of hemoglobin A which, on staining with Amidoschwartz, was similar in width and intensity to the A₂ in the more concentrated hemolysates. Comparison of the two bands permitted a crude estimate of the amount of A₂. Intermediate concentrations (0.5 mg. of hemoglobin per ml.) also required staining with Ami-

doschwartz, but resolution of hemoglobins A, C, and S was improved (Fig. 5), and hemoglobin F became distinguishable from hemoglobin A. These electrophoretic methods therefore seem to lend themselves to technical compromises

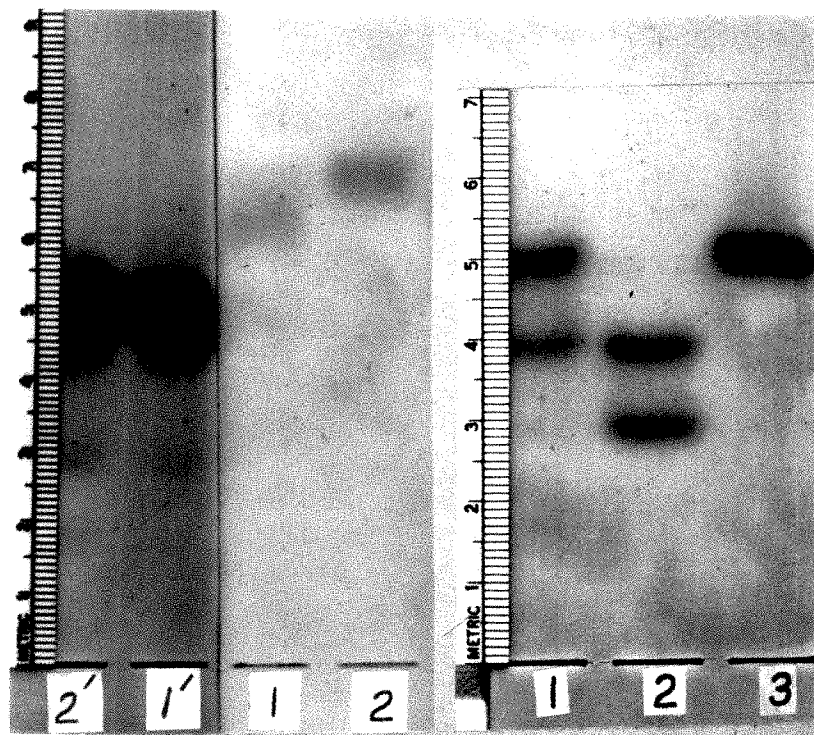


FIG. 4. (Left) Relative positions of glucose-6-phosphate dehydrogenase and hemoglobins A and A² after vertical starch-gel electrophoresis. Channels 1 and 2 are identical to those of Fig. 2. Channels 1' and 2' represent the opposite half of the gel stained with Amido-schwartz.

FIG. 5. (Right) Vertical starch-gel electrophoretic patterns of hemoglobins A, C, and S. Channel 1, hemoglobins A-S. Channel 2, C-S. Channel 3, hemoglobin A.

which might permit simultaneous identification of several genetic characteristics in surveys requiring only finger-prick samples of blood and no direct enzymic assay.

SUMMARY

Further details and statistical analysis are presented of a study of electrophoretic differences in glucose-6-phosphate dehydrogenase of American Negroes. Hemolysates from finger-prick blood of 343 persons, principally mothers and sons, were subjected to vertical starch gel electrophoresis and specific staining for the enzyme. Samples from 291 of these subjects were read under blind conditions. The data suggest sex-linked inheritance of the electrophoretic differences and allow rejection of each of several proposed autosomal models. Although the findings fit a two-locus model well, they do not permit exclusion of triple allelism at a single locus. The electrophoretic techniques can be modified

to allow simultaneous determination of several inherited characteristics of hemolysates.

ACKNOWLEDGMENTS

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APPENDIX

Table 4 presents models of two autosomal, sex-modified alleles, c_1 and c_2 , determining the electrophoretic difference, and of two sex-linked alleles, d_1 and d_2 , the latter determining primaquine sensitivity. Gene frequencies and variances were estimated by the method of maximum likelihood (Kempthorne, 1957). Autosomal models 1a and 4a to 4d could be rejected because the estimated gene frequencies of c_2 differed significantly in mothers and sons. Models 1b and 2a required an estimate of d_2 that seemed too high to allow the prevalence (8 to 10 per cent) and penetrance previously observed for primaquine sensitivity in Negro males. In addition, model 1b allowed a high expectancy of B sons from A mothers. In contrast, models 3a to 3c provided a similar estimate of gene frequencies in mothers and sons (table 5); and model 3a could fit the mother-son data rather closely, presenting exceptions to apparent sex-linked inheritance in only 9 per cent of mother-son pairs (table 6). But 3a to 3c required a significantly higher c_2 in fathers than in sons: 71 B sons and no A sons were produced by 71 B mothers, whereas the expected ratio of A:B sons would be equal to, or greater than, the $c_1:c_2$ ratio in fathers. The $P = .05$ confidence limit ($C_{\frac{71}{2}} = .05$) for the paternal c_2 is .958, more than four S.D.'s above the estimated c_2 of sons.

Table 7 presents models of two alleles at each of two loci on X chromosomes. One of these, 6a, fits the data better than any of the other tested models; maximum likelihood estimates of gene frequencies in sons provided excellent agreement with phenotypes in mothers and with the mother-son array of phenotypes (table 6). One array of five classes, in which expectancies of less than 6 were pooled (table 9), provided a fit (Kempthorne, 1957) with a χ^2 of 1.27, d.f. 2, $P > .05$, even with the gene frequencies estimated only from sons. This model would simulate triple allelism (Model 7) very closely: three phenotypes could be observed among the sons of only 9 per cent of the mothers, those with genotype $c_1c_2d_1d_2$; an examination of over 390 individuals could be required to reject this model from a study of randomly selected mothers and two sons of each mother, and over

TABLE 4. MODELS OF TWO LOCI, ONE AUTOSOMAL (C) AND ONE SEX-LINKED (D), WITH SEX MODIFICATION IN MALES

c genotype d genotype Model No.	Males						Females					
	c ₁ c ₁ d ₁	c ₁ c ₂ d ₁	c ₁ c ₂ d ₁	c ₁ c ₁ d ₂	c ₁ c ₂ d ₂	c ₂ c ₂ d ₂	c ₁ c ₁ d ₁ d ₁	c ₁ c ₂ d ₁ d ₁	c ₂ c ₂ d ₁ d ₁	c ₁ c ₁ d ₁ d ₂	c ₁ c ₂ d ₁ d ₂	c ₂ c ₂ d ₁ d ₂
1 a	B	B	A	B	A-	A-	B	AB	A	B	AB	A-
1 b	B	B	A	B	B	A-	B	AB	A	B	AB	A-
2 a	B	A	A	B	A-	A-						
2 b	B	A	A	B	A	A-						
3 a	A	A	B	A	A-	A-	A	AB	B	A	A	A-
3 b	A	A	B	A	A-	A-	A	AB	B	A	A	A-
3 c	A	A	B	A	A-	A-	A	AB	B	A	A	A-
4 a	A	B	B	A	A-	A-	A	AB	B	A	A	A-
4 b				Same			A	AB	B	A	A	A-
4 c				Same			A	AB	B	A	A	A-
4 d				Same			A	AB	B	A	A	A-

TABLE 5. MAXIMUM LIKELIHOOD ESTIMATES OF GENE FREQUENCIES IN AUTOSOMAL MODELS

Model No.	Gene Frequencies		Variances		Standard Deviations		Classes Used in Estimations
	c_1	c_2	d_1	d_2	c_2	d_2	
1 a	.479 .763	.521 .237	.846 .647	.154 .353	$c_2, 14.8 \times 10^{-4}$ $c_2, 6.63 \times 10^{-4}$	$c_2, 0.038$ $c_2, 0.026$	3 phenotypes in sons 4 phenotypes in mothers
1 b	.590	.410	.659	.341			3 phenotypes in sons
2 a	.769	.231	.660	.340			3 phenotypes in sons
2 b							
3 a	.139 .135	.861 .865	.879 .891	.121 .109	(No max. likelihood for $0 < d_2 < 1$) $c_2, 5.27 \times 10^{-4}$ $d_2, 8.02 \times 10^{-4}$ $c_2, 36.2 \times 10^{-4}$ $d_2, 35.7 \times 10^{-4}$	$c_2, 0.023$ $d_2, 0.028$ $c_2, 0.061$ $d_2, 0.060$	3 phenotypes in sons 3 phenotypes in sons 4 phenotypes in mothers
3 b	.221	.779	.958	.042	Same values as 3a for $c_2, 6.75 \times 10^{-4}$ $d_2, 5.80 \times 10^{-4}$	$c_2, 0.026$ $d_2, 0.024$	3 phenotypes in sons 4 phenotypes in mothers
3 c	.122	.878	.846	.154	$c_2, 4.26 \times 10^{-4}$ $d_2, 12.5 \times 10^{-4}$	$c_2, 0.021$ $d_2, 0.035$	3 phenotypes in sons
4 a, b	.521	.479	.846	.154	Same as 3a or 3b for $c_2, 13.7 \times 10^{-4}$ $d_2, 12.5 \times 10^{-4}$	$c_2, 0.037$ $d_2, 0.035$	4 phenotypes in mothers 3 phenotypes in sons
4 c	.192	.808	.932	.068	Same as 3a or 3b for $c_2, 9.3 \times 10^{-4}$ $d_2, 7.6 \times 10^{-4}$	$c_2, 0.031$ $d_2, 0.028$	4 phenotypes in mothers 4 phenotypes in mothers
4 d	.286	.714	.805	.195	$c_2, 11.3 \times 10^{-4}$ $d_2, 25.3 \times 10^{-4}$	$c_2, 0.034$ $d_2, 0.050$	4 phenotypes in mothers

170 individuals could be required in a study of three sons of each mother. A distinction between this model and triple allelism may not be obtained readily even from observations of electrophoretic phenotypes of leukocytes, for activity of G-6-PD in primaquine sensitive Negroes is less impaired in leukocytes than in red cells (Marks, Gross and Hurwitz, 1959). Because of this effect, the genotype $c_2^s d_1 d_2$ could be expressed in leukocytes as AB. Such a model would provide a clue of non-allelism in, for instance, only 0.4 per cent of families in which both parents and one daughter are tested.

The data may also fit another model of two sex-linked loci, such as 6b; but they will not fit model 5a, in which an allele simply converts A to A-.

A model of three alleles at one sex-linked locus appears in table 8. The presence of A- sons from B mothers is incompatible with triallelism unless the faint A- band were sometimes missed in hemolysates of A-B mothers. Evidence has been presented that this may occur. Yet even if an allowance is made that 66 per cent of A-B samples would be called B, a relatively poor fit is obtained for triple allelism (model 7). The five classes shown in table 9 provide a test of major discrepancies while minimizing the misclassification of A-B. Maximum likelihood estimates of the three gene frequencies from this array provide a fit (Kempthorne, 1957) with a χ^2 of 6.43 (2 d.f., P of 0.04). If the log likelihoods from this array are added to that of the supplementary group (table 1 of text and table 9, bottom row), however, a fit with a χ^2 of 5.17 (2 d.f., P of 0.07) is obtained. These tables, moreover, exclude the discordant reading on three sons and their mothers, all possibly B sons of AB women. A return of any of these to table 1 would reduce the χ^2 . Thus, the findings do not permit rejection of sex-linked triple allelism.

TABLE 6. COMPARISON OF THE FIT OF SEVERAL MODELS TO OBSERVATIONS

The numbers in each group represent, from top to bottom, the observed data; the fit from gene frequencies estimated by autosomal model 3a from mothers; the fit from gene frequencies estimated by model 6a from sons; and the fit from gene frequencies estimated from the 5 classes of model 7.

	B	Mothers AB	A	A-	Totals
Sons	B	71 69.3 67.8 74.7	17 19.3 20.2 20.9	0 1.4 0 0	0 0 0 0
	AB	0	0	0	0
	A	0 10.9 0 0	25 15.5 23.3 17.4	6 2.8 7.9 7.1	0 0 0 0
	A-	8 0 10.4 6.9	6 9.8 3.1 3.5	1 4.7 0.6 2.7	1 1.2 1.9 1.6
	Totals	79 80.2 78.2 81.6	48 44.6 46.6 41.8	7 8.9 8.5 9.8	1 1.2 1.9 1.6
					135

TABLE 7. MODELS FOR TWO LOCI, BOTH SEX-LINKED

		Males				Females					
c genotype	d genotype	c ₁ d ₁	c ₂ d ₁	C ₁ d ₂	c ₂ d ₂	c ₁ c ₁ d ₁ d ₁	c ₁ c ₂ d ₁ d ₁	c ₂ c ₂ d ₁ d ₁	c ₁ c ₁ d ₁ d ₂	c ₁ c ₂ d ₁ d ₂	c ₂ c ₂ d ₁ d ₂
Model No.											
5 a	A	B	A ⁻	B	AB	B	AB	A	B	B	A ⁻
5 b	A	B	A ⁻	B	AB	B	B	A	B	B	A ⁻
6 a	B	A	A ⁻	A	AB	A	AB	B	A	A	A ⁻
6 b	B	A	A ⁻	A	AB	A	AB	B	A ⁻	A ⁻	A ⁻

TABLE 8. MODEL OF ONE SEX-LINKED LOCUS, THREE ALLELES

		Males				Females			
genotype	Model No.	b	a	a ⁻	bb	ab	au	a-b	a-a
7		B	A	A ⁻	B	AB	A	34% AB 66% B	A A ⁻

TABLE 9. MAXIMUM LIKELIHOOD ESTIMATES OF GENE FREQUENCIES IN SEX-LINKED MODELS

Model No.	c ₁	Gene frequencies c ₂	d ₁	d ₂	Variances	Standard Deviations	Classes Used in Estimations
5 a	.652 .720	.348 .280	.660 .634	.340 .366	(Goodness of fit: $\chi^2 = 7.86$, P of 0.002, 2 d.f.)		3 phenotypes in sons B/B*, B or AB/A-, AB/B, AB/A, A or A-/A or A-
5 b					Same as 5a for		3 phenotypes in sons
6 a	.2296	.7704	.8462	.1538	(Goodness of fit: $\chi^2 = 1.27$, P > .5, 2 d.f., when applied to the 5 classes of 5a)		3 phenotypes in sons
6 b	.2602	.7398	.8815	.1185	(Goodness of fit: $\chi^2 = 2.86$, P of 0.24, 2 d.f., when applied to the 5 classes of 5a)		3 phenotypes in sons
7	$\frac{a}{.230}$	$\frac{a-}{.119}$	$\frac{b}{.651}$		a, 13.1×10^{-4} a-, 5.3×10^{-4} b, 16.8×10^{-4} a, 11.0×10^{-4} a-, 28.3×10^{-4} b, 10.8×10^{-4} (Goodness of fit: $\chi^2 = 6.43$, P of 0.04, 2 d.f.)	a, 0.036 a-, 0.023 b, 0.041 a, 0.033 a-, 0.053 b, 0.033	3 phenotypes in sons
	.203	.076	.721				4 phenotypes in mothers
	.182	.109	.709				5 classes of 5a
	.184	.110	.706		(Goodness of fit: $\chi^2 = 5.17$, P of 0.07, 2 d.f.)		5 classes of 5a, likelihoods pooled with those of table 1, lowest row†

*Phenotype of mother/phenotype of son.
†In table 1 (text), lowest row, expectancy of B or AB/A- is 7b and the expectancy of A or A-/A- is 7 (a + a-).

A Syndrome of Deaf-Mutism Associated with Albinism Showing Dominant Autosomal Inheritance

WALTER TIETZ

*Department of Pediatrics, Southern California
Permanente Medical Group, Fontana, California*

THE ASSOCIATION of deafness and albinism has been known for some time. Recently, renewed interest in this association has been revived by the publication of various pedigrees of the Waardenburg syndrome (Waardenburg, 1951). It is the purpose of this communication to present a unique pedigree in which deaf-mutism and albinism are dominantly inherited and constantly associated.

My attention was first focused on the association of albinism and deaf-mutism when, in the course of a routine pediatric practice, I examined an obviously albino newborn infant. The father insisted that this newborn was also deaf. Clinical examination confirmed the parent's impression. My curiosity was aroused and a more detailed genetic history was obtained. All affected members of this pedigree exhibit the following features:

1. Albinism is generalized throughout the body but does not involve the eyes. The irides are blue and there are no associated ocular abnormalities such as nystagmus or photophobia. No one in this family exhibits lateral displacement of the medial canthi of the eyes, a prominent feature of the Waardenburg syndrome. The type of albinism exhibited by members of this family is one in which melanin is absent in the skin and hair bulb, but present in the iris. It has been called albinoidism (Cockayne, 1933) to distinguish it from the usual albinism.
2. Complete deaf-mutism is present in all affected members of this pedigree. Audiometric testing of two members confirmed a nerve deafness.
3. All the involved individuals have hypoplasia of the eyebrows with only a few sparse albino hairs where the eyebrows normally are.

Three affected members of this family were given a complete physical examination.

J. F., the propositus, is a 3½ year old male albino whose physical and mental development has been completely normal except for the presence of deaf-mutism. His deafness was first suspected during infancy because of lack of response to loud noises, and has subsequently been confirmed by clinical observation. The child has light blond hair, blue irides, and typical albino skin throughout. Ophthalmological examination disclosed no refractive error. There was no nystagmus and no photophobia. Slit lamp examination was normal, as was the examination of the fundi. There was no evidence of ocular albinism. The distance between the medial canthi was 36 mm., between the lateral canthi 68

mm., and the interpupillary distance was 50 mm. The punctum was 3 mm. from the medial canthus.

R. F., 7 year old brother of the propositus, is an alert, intelligent child who has all the features of his 3½ year old brother. He has a fraternal twin who is unaffected. R. F. has light blond hair, typical albino skin throughout, and blue irides. An audiogram was done which revealed 100 decibel loss in both bone and air conduction in all frequencies. The child was seen by our ophthalmologist, and no refractive error was found. There was no evidence of nystagmus or photophobia. The irides were blue and slit lamp examination was normal. No abnormalities were noted on fundusoscopic examination, and there was no evidence of ocular albinism. The distance between the medial canthi was 35 mm., between the lateral canthi 78 mm., and the interpupillary distance was 57 mm. The punctum was 3 mm. from the medial canthus.

The mother of the propositus is a 33 year old deaf-mute who appears to be



FIG. 1. Family picture showing normal father and affected mother who is holding the affected propositus. There are four other children, one of whom, the boy on the left, is also affected.

bright and alert. Physical examination revealed a well developed, well nourished female having light blond hair, blue eyes, and typical albino skin throughout. Two separate skin biopsies were performed, neither of which revealed the presence of melanin. Audiometric examination disclosed 100 decibel loss in both bone and air conduction at all frequencies. This pattern was seen in both ears and was interpreted by our audiometrist as a pattern consistent with nerve damage. The patient was examined by our ophthalmologist and was found to have a mild refractive error which was easily remedied by corrective glasses. There was no nystagmus and no photophobia. The irides were blue, and no abnormalities were noted by slit lamp examination. The fundi were normal, and there was no evidence of ocular albinism. The distance between the medial canthi was 34 mm., between the lateral canthi 84 mm., and the interpupillary distance was 63 mm. The punctum was 4 mm. from the medial canthus.

The maternal grandmother is living and has all the features of the affected individuals of this pedigree. She verified the information on the older, deceased members of the pedigree. She is quite alert and was able to give a consistent and reliable history. No attempt was made to directly examine the affected siblings of the mother of the propositus.

Fig. 1 is a photograph of the propositus and his parents and sibs. The propositus has four sibs and only one of these is affected. The affected brother has a fraternal twin who is normal. The mother and four maternal aunts are affected. The maternal grandmother is living and is an affected individual. As can be seen from Fig. 2, this pedigree has 14 affected individuals, six males and eight females. All show the features of the syndrome; furthermore, individuals having these features are present in each generation.

Thus, it is quite clear that the features exhibited by affected members of this pedigree are inherited in a dominant and autosomal fashion. The penetrance in this family is complete. At the time this family was presented at a meeting of the American Society of Human Genetics (Tietz, 1960), a search of the literature revealed no pedigree similar to this family. Since then, Margolis (1962) has described a family showing similar features but with clearly sex linked inheritance.

DISCUSSION

The association of albinism with deaf-mutism has been described, but this association is not a constant one. In 1951 Waardenburg described a syndrome which drew attention to this association and stimulated interest in it. The syndrome described by Waardenburg has the following features:

1. Lateral displacement of the inner canthi of the eye (Dystopia canthorum).
2. High, broad nasal root.
3. Confluence of the eyebrows with hypertrichosis of the medial parts.
4. Partial or total heterochromia iridum.
5. White forelock.
6. Unilateral or bilateral congenital deafness.

Waardenburg concluded that the syndrome has a dominant autosomal mode of

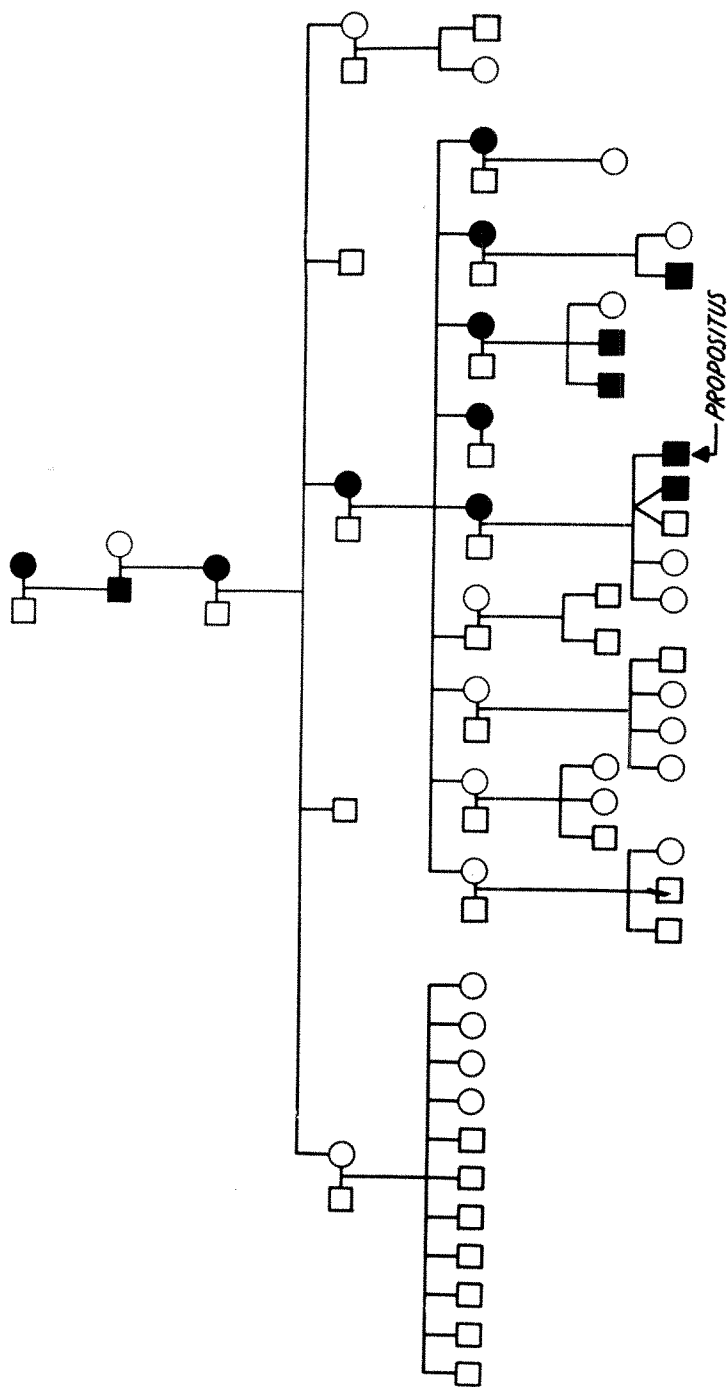


FIG. 2. Pedigree showing 14 affected individuals, six males and eight females. Affected individuals are colored black in the diagram. There are no partially affected individuals and no generation is skipped.

inheritance and that each of its components has a variable penetrance. Among 161 affected persons from 14 families, he found that 99 per cent had dystopia canthorum, 78 per cent broad nasal root, 45 per cent confluent eyebrows, 25

per cent heterochromia iridum, 20 per cent congenital deafness and 17 per cent white forelock. Since the original report, further cases of this syndrome have been described, mainly involving northern Europeans. Recently DiGeorge, Olmstead and Harley (1960) reported a family of Negroes with this syndrome from the United States. They suggested a number of additional features of the Waardenburg syndrome among which they listed abnormal depigmentation of the skin, premature graying of the hair, a characteristic facial appearance and pigmentary changes of the fundi. A pathological report of a case of Waardenburg syndrome reported in 1959 (Fish) showed absence of the organ of Corti and atrophy of the spiral ganglion and nerve.

In 1926 Mende described a family in which deaf-mutism was inherited as an irregular dominant and was associated in the last two generations with white forelock, eyebrows and eyelashes and white spots on the skin. Waardenburg felt that photographs of Mende's family showed dystopia of the canthi. This is a cardinal feature of the syndrome described by Waardenburg (Waardenburg, 1951).

In 1962 Margolis described a pedigree that comprised more than 100 persons in six generations, with only 14 males affected. The affected males exhibited deaf-mutism associated with total albinism. This pedigree clearly showed a sex-linked inheritance pattern.

The etiology of deaf-mutism is complex, inasmuch as the acquired cases must be separated from those of congenital origin. This must always be remembered when analyzing the inheritance of deaf-mutism. Dahlberg (1931), using extensive figures from previous authors, presented a statistical analysis of congenital deaf-mutism and concluded that most cases are due to recessive inheritance. He also felt that more than one recessive factor may be involved and that there are at least three dominant forms of congenital deaf-mutism.

Recently, in a monograph edited by Kallman, Sank (1962) presented preliminary data which showed that about 25 per cent of the cases of deaf-mutism are dominantly inherited. Also, the data presented by Sank seemed to implicate more than one recessive gene when recessive inheritance occurred. Furthermore, in the dominant form of deaf-mutism, the expressivity pattern seemed to be more variable. Likewise, the Waardenburg syndrome, which is dominantly inherited, has a low penetrance for deafness.

Similarly, albinism in man is usually inherited as a recessive trait. The inheritance of albinism is very thoroughly covered in Cockayne's textbook (Cockayne, 1933). In this book a few pedigrees with dominant form of albinism are cited. Clinically, they are albinoid and can be distinguished from the true albinos by the absence of the usual ocular defects. Also, the hair and skin of the albinoid are slightly darker than those of albinos, and the texture of the hair is not so fine. The term albinoid was used by Haecker, as cited by Cockayne (1933). Haecker described pedigrees of three families with dominant albinism. It should be mentioned that in all described families with albinoidism, the dominance is incomplete or irregular. Thus, there have been very few described cases of dominant albinism, most albinos being true recessives.

In the family here described, there are two phenotypic effects, albinism and

deaf-mutism. Each of these shows a dominant form of inheritance, and they are constantly associated. These effects have caused some interesting speculations concerning pathogenesis. Both pigment cells and spiral ganglion cells arise from the neural crest. Fish (1959) attempted to relate the abnormalities of Waardenburg's syndrome to a genetic defect of the neural crest. Margolis (1962) suggested a similar mechanism in his pedigree. A different genetic defect in these cells may account for the findings in the present pedigree. It should be kept in mind that these are all speculations at present.

SUMMARY

A large pedigree has been presented where there is a constant association of albinism with blue eyes, deaf-mutism, and hypoplasia of the eyebrows. This syndrome shows dominant inheritance, and penetrance appears to be complete. The features exhibited by this pedigree appear to be unique and different from the previously described associations of albinism and deaf-mutism.

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Hemoglobin Pôrto Alegre, a Possible Polymer of Normal Hemoglobin in a Caucasian Brazilian Family

CASEMIRO V. TONDO¹, FRANCISCO M. SALZANO¹ AND DONALD L. RUCKNAGEL²

¹*Departamento de Genética, Instituto de Ciências Naturais, Universidade do Rio Grande do Sul, Pôrto Alegre, Rio Grande do Sul, Brazil, and* ²*Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan*

A LARGE NUMBER OF inherited anomalies of human hemoglobins have been reported since the introduction of electrophoretic techniques into medicine (reviewed by Rucknagel and Neel, 1961). As a consequence of the application of these techniques to the study of undiagnosed anemias, the earlier abnormalities detected, hemoglobins S, C, D, E, and H, were associated with hematologic disease. Most of the more recently detected abnormal hemoglobins have been discovered during population surveys and thus are unaccompanied by clinical or hematologic abnormalities. In this report a new asymptomatic hemoglobin anomaly, previously designated hemoglobin Pôrto Alegre (Tondo, Salzano and Rucknagel, 1961; Rucknagel, Tondo and Salzano, 1962), is described in a Caucasian Brazilian family of Portuguese origin, ascertained during a population survey. Despite unusual physicochemical properties, it apparently confers no disadvantage upon individuals possessing the gene.

Hemoglobin P.A. came to our attention because of the appearance on paper electrophoresis at alkaline pH of a large amount of a component migrating slightly anodally to hemoglobin A, similar to Hb A₃. On starch block electrophoresis at pH 8.6 no unique abnormalities were noted in the hemolysates of the heterozygotes. On starch gel electrophoresis at the same pH an additional component was visible, migrating much slower than hemoglobin A, between hemoglobins S and C. This retarded mobility in starch gel led to other investigations that suggested that hemoglobin P.A. is a polymer of normal hemoglobin.

MATERIALS AND METHODS

Venous blood was collected in balanced oxalate anticoagulant and the erythrocytes separated and washed with 0.15 M NaCl. After lysis of the erythrocytes by the addition of water, and freezing and thawing, the stroma was removed by centrifugation. The resulting solution was adjusted colorimetrically to give a concentration of 8 gm. of hemoglobin per 100 ml.

Paper electrophoretic separations were made in a Durrum cell or by pressing

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the paper between glass plates, using Whatman 3 MM filter paper and Veronal buffer at pH 8.8 and ionic strength 0.05. The papers were stained with bromophenol blue. The relative concentrations of components were determined by densitometry with the Spinco Analytrol before staining the strips. Symmetrical Gaussian curves were drawn and the method of Tiselius and Kabat (1939) applied.

Vertical starch gel electrophoresis was performed at pH 8.6 with 0.03 M borate buffer by the method of Smithies (1959), inserting filter paper squares saturated with hemolysate into incisions in the gel. Prior to application to the gel one drop of 2 per cent potassium cyanide, neutralized with 10 per cent acetic acid and indicator paper, was added to approximately 0.3 ml. of the hemolysate to convert any methemoglobin present to the cyan-met form. After electrophoresis longitudinally sliced halves of the gels were stained with ortho-dianisidine and amidoschwartz stains. Starch block electrophoresis was performed in barbital buffer, pH 8.8 and ionic strength 0.06. This was executed both by covering the block with thin cellophane (Saran Wrap, Dow Chemical Company) as described by Kunkel *et al.* (1957) or by electrophoresing in the humidity chamber as described by Gerald and Diamond (1958). Agar gel electrophoresis was performed at pH 6.5 for 5 hours using a modification of the technique of Robinson *et al.* (1957). The hemoglobin absorption spectra were determined in 0.1 ionic strength phosphate buffer at pH 6.8 using the Beckman model DK-2 ratio recording spectrophotometer. Solubility in 2.58 M phosphate buffer was measured by the method of Itano (1953). Fetal hemoglobin determinations were performed by the method of Singer, Chernoff and Singer (1951).

The blood groups were determined using the following reagents: anti-A, -B, -M, -C, -c, -D, -E, A₁ from Ortho Pharmaceutical Co.; anti-A₂: seed extracts of *Ulex europeus* prepared following the method of Boyd and Shapleigh (1954); anti-N: Ortho and seed extracts of *Vicia graminea* prepared according to Ottensooser (1958). All tests were performed in tubes, the instructions indicated by the supplier of each particular serum being followed rigorously. In all cases the two anti-N cited were used simultaneously for a mutual control of specificity.

RESULTS

Clinical Observations

The propoſita's blood was collected during the course of population studies to determine the prevalence of abnormal hemoglobins in Pôrto Alegre, Brazil. She is a healthy 10-year-old Caucasian girl (V-100, Fig. 3) whose medical history was unremarkable. Physical examination was within normal limits; the liver and spleen were not palpable. Seventy-six other members of her family have been studied; all of them are white. Some live in Pôrto Algŕe but the majority inhabit the neighboring counties of Sâo Leopoldo, Novo Hamburgo, and Rolante.

A detailed hematological examination was performed on V-100, the propoſita. Erythrocytic indices, which are normal, are presented in table 1 along with those of three other relatives studied. Additional determinations on her blood are

TABLE 1. HEMATOLOGIC VALUES OF SELECTED INDIVIDUALS

	V-81	IV-6	V-100	V-28
Age	28	54	10	17
Sex	M	M	F	F
Hb genotype	P.A./P.A.	A/P.A.	A/P.A.	A/A
Hemoglobin (gm. %)	14.9	15.5	11.6	14.0
Hematocrit	43	44	36	40
R. B. C. $\times 10^6/\text{mm}^3$	4.8	5.1	4.2	4.5
Mean cell volume (μ^3)	90	86	86	89
Mean cell Hb concentration (%)	35	35	32	35
Mean cell Hb γ/cell	31	30	28	31
Erythrocyte morphology	Normal	Normal	Normal	Normal

as follows: leukocytes, 8,000 per mm^3 ; differential count (in per cent): lymphocytes, 45; monocytes, 5; segmented neutrophils, 44; bands, 2; eosinophiles, 4; color index, 0.9; platelets, 360,000 per mm^3 ; reticulocytes, 1 per cent; mean corpuscular volume (M.C.V.), $86\mu^3$; mean corpuscular hemoglobin (M.C.H.), 28 $\mu\text{g.}$ per cell; mean corpuscular hemoglobin concentration (M.C.H.C.), 32 per cent; saturation index, 0.9; volume index, 0.9; slight anisocytosis and poikilocytosis judged to be within normal limits; erythrocyte sedimentation (Westergren) in one hour, 18 mm.; in 2 hours, 32 mm.; Katz index, 17; direct bilirubin, 0; indirect bilirubin, 0.2 mg. per cent; serum iron, 0.107 mg. per cent; osmotic fragility, normal.

All the other members of the family, homozygotes or heterozygotes for the gene under discussion, appeared healthy; no systematic clinical studies were performed. However, to exclude the presence of thalassemia, blood smears were made of IV-6 (another heterozygote) and V-81 (a homozygote). As shown in table 1, the erythrocytic indices and morphology proved to be normal.

On paper electrophoresis at pH 8.6, the unstained pattern of the *proposita's* hemoglobin was essentially normal, like that of V-84 of Fig. 1, with an apparent increase in the hemoglobin A_3 component. After staining with bromphenol blue, a minor component could be seen migrating slightly cathodally but not separating clearly from hemoglobin A. On starch gel electrophoresis, however, a distinct component, constituting approximately 20 per cent of the total hemoglobin, was visible in the region between Hb C and Hb S, closer to the latter, as is the case in V-84 in Fig. 2. The Hb A_3 region was more dense than in normal hemolysates of comparable age and appeared to increase with aging of the specimen. The remainder of the hemoglobin appeared to be Hb A. This pattern was observed in 18 other members of the family.

In six individuals the slow component visible on starch gel electrophoresis was estimated to comprise approximately 75 per cent of the hemoglobin (Fig. 2b). In these Hb A was either absent or amounted to less than 10 per cent of the total, but the "fast" component with the mobility of Hb A_3 was in all cases still visible, located slightly anodally to the position of hemoglobin A, and amounting to 10 to 15 per cent of the hemoglobin when measured by starch block electrophoresis.

Fetal hemoglobin levels in 67 individuals, including the 19 individuals with minor amounts of Hb P.A. and six with predominantly Hb P.A., varied between .2 and .4 per cent (table 2). The solubility of the relatively pure Hb P.A. in

2.58 M phosphate buffer was 1.3 Gm. per L., within our normal range of 1.2 to 1.5.

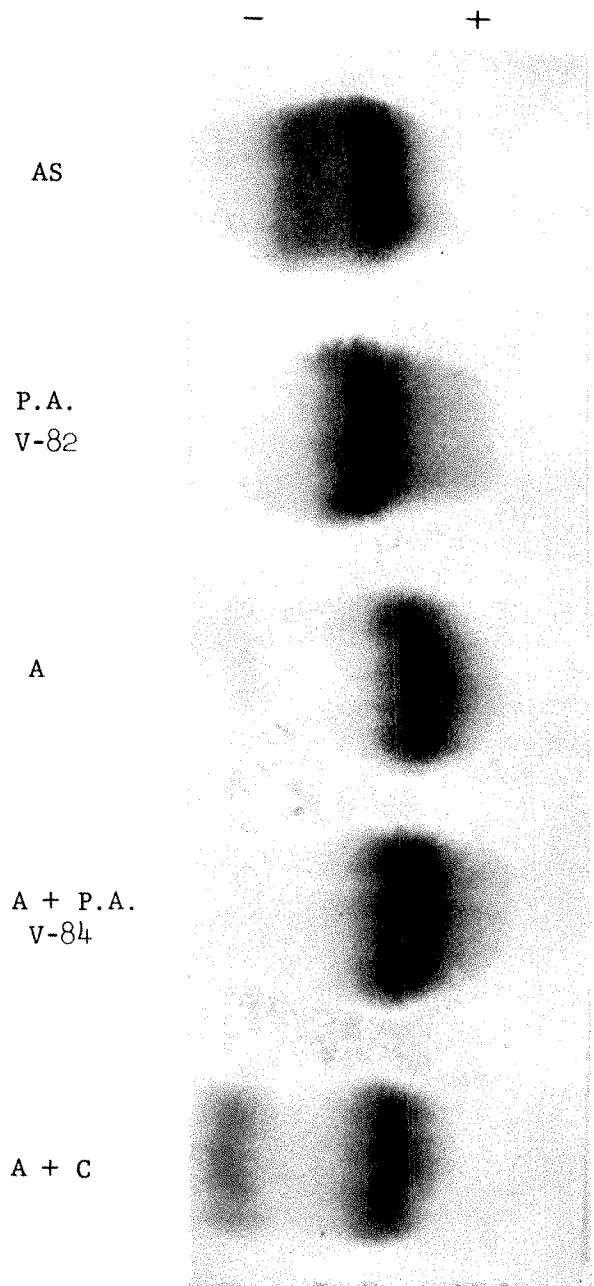


FIG. 1. Paper electrophoresis performed by pressing Whatman 3 MM paper between glass plates. Veronal buffer, pH 8.6, ionic strength 0.05. Bromphenol blue stain.

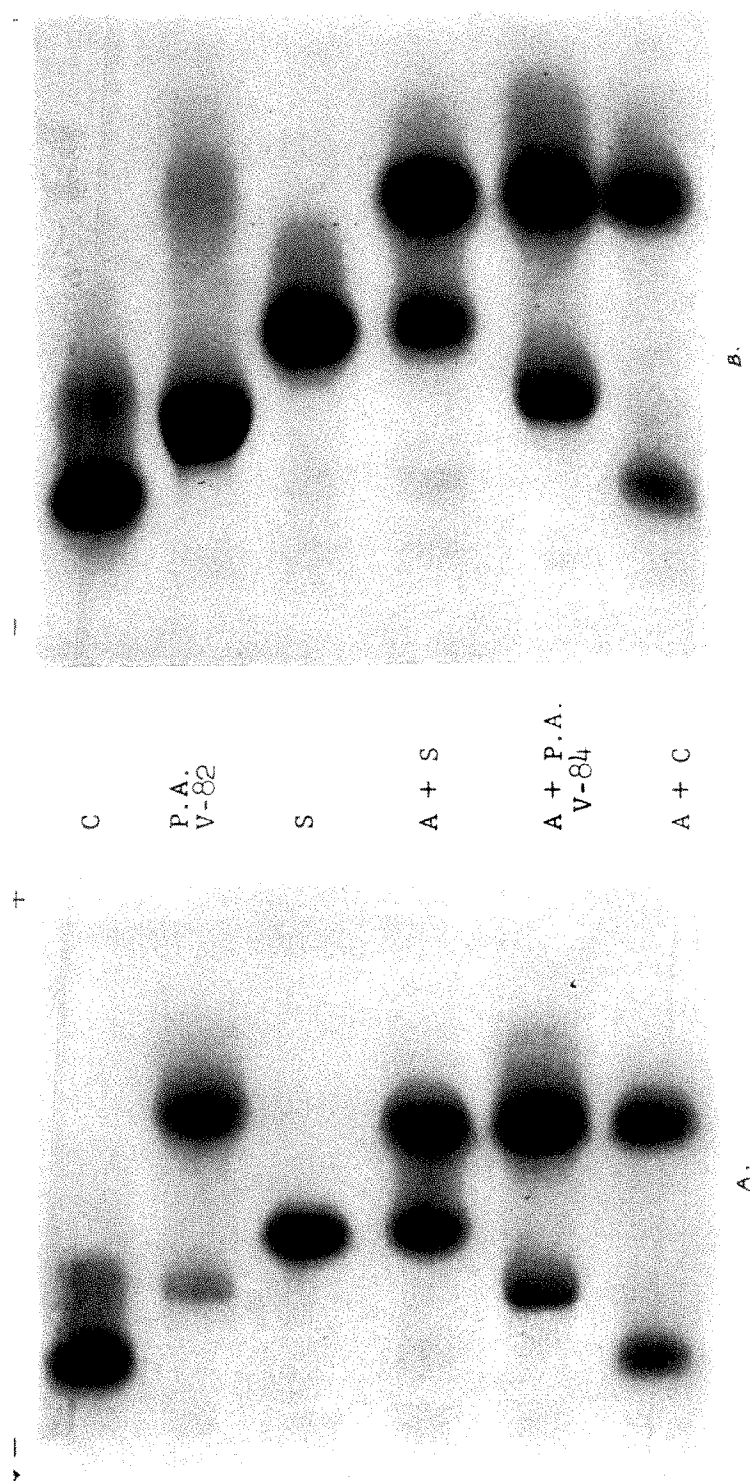


FIG. 2. Comparison of Hb P.A. homozygote (V-82) and heterozygote (V-84) with known hemoglobins on vertical starch gel electrophoresis, using 0.03 M borate buffer, pH 8.6, and stained with amido-schwartz. a. Freshly prepared hemolysates of Hb P.A. b. Hemolysates stored at 4°C for 6 weeks.

TABLE 2. BLOOD GROUPS AND HEMOGLOBINS

Reference Fig. 3	Components					
	Sex	Hb genotype	% Hb F	ABO	Blood groups MN	Rh
IV-1	M	A/A	—	A ₂	M	CcDee
V-2	M	A/A	—	O	MN	CcDee
IV-2	F	A/P.A.	0.3	A ₂	M	ccddee
V-14	F	A/P.A.	0.3	A ₁	MN	CcDee
IV-4	M	A/A	0.3	A ₂	N	CcDee
V-20	F	A/A	0.3	B	MN	CcDee
VI-9	F	A/A	0.3	A ₂	M	CcDee
VI-11	F	A/A	0.3	B	MN	CCDee
VI-13	F	A/A	0.3	B	MN	CCDee
VI-14	F	A/A	0.3	A ₂	M	CCDee
V-22	F	A/A	0.3	B	MN	CcDee
VI-22	F	A/A	—	A ₁ B	N	CcDee
VI-23	F	A/A	0.3	B	MN	CcDee
V-24	F	A/A	0.4	B	MN	CcDee
V-25	F	A/A	0.4	O	N	CCDee
IV-5	M	A/P.A.	0.4	A ₂	MN	CcDee
V-26	F	A/P.A.	0.3	A ₂	N	CCDee
V-27	F	A/P.A.	0.3	A ₂	N	CcDee
V-28	F	A/A	0.2	A ₂	MN	CcDee
V-29	M	A/A	0.4	O	N	CcDee
IV-6	M	A/P.A.	0.4	O	MN	CcDee
w.IV-6	F	A/A	0.2	O	M	CCDee
V-32	F	A/P.A.	0.3	O	MN	CCDee
V-34	F	A/P.A.	0.3	O	MN	CcDee
V-35	M	A/P.A.	0.3	O	M	CCDee
V-36	M	A/P.A.	0.3	O	MN	CcDee
V-37	F	A/P.A.	0.3	O	MN	CCDee
V-38	F	A/A	0.4	O	M	CCDee
V-39	F	A/A	0.4	O	MN	CcDee
IV-8	M	A/P.A.	0.3	A ₂	N	ccddee
2nd w.IV-8	F	A/A	0.2	O	N	CcDE
V-49	M	A/A	0.3	A ₂	N	ccDE
V-50	F	A/P.A.	0.3	A ₂	N	ccDE
V-52	F	A/A	0.3	A ₂	N	CcDee
V-53	M	A/A	0.4	O	N	ccDE
V-55	M	A/A	0.3	O	N	ccDE
V-56	M	A/A	0.3	O	N	CcDee
IV-9	M	A/A	0.4	A ₂	MN	ccddee
w.IV-9	F	A/A	—	O	MN	CCDee
V-58	M	A/A	0.3	O	N	CcDee
V-60	F	A/A	0.3	A ₂	MN	CcDee
V-61	M	A/A	0.4	A ₂	N	CcDee
V-62	M	A/A	—	A ₂	M	CcDee
V-63	F	A/A	0.3	A ₂	M	CcDee
V-64	M	A/A	0.4	A ₂	MN	CcDee
V-65	F	A/A	0.3	A ₂	N	CcDee
V-66	M	A/A	0.4	A ₂	MN	CcDee
V-67	M	A/A	0.3	O	N	CcDee
IV-11	M	P.A./P.A.	0.3	O	MN	ccddee
IV-12 (w.IV-11)	F	A/P.A.	0.3	A ₂	M	CcDee
V-81	M	P.A./P.A.	0.3	O	MN	ccDee
V-82	M	P.A./P.A.	0.3	A ₂	MN	CcDee
V-83	M	A/P.A.	0.3	O	M	ccDee
V-84	M	A/P.A.	0.3	O	M	CcDee
V-85	M	P.A./P.A.	0.3	A ₂	MN	CcDee
V-86	F	P.A./P.A.	0.3	A ₂	MN	ccDee
V-87	F	A/P.A.	0.3	O	MN	ccDee
V-88	F	P.A./P.A.	0.3	O	M	CcDee

2nd h.IV-14	M	A/A	—	O	—	D
V-97	M	A/A	0.4	A ₂	MN	CcDee
V-98	F	A/A	0.3	A ₂	MN	CcDee
V-99	M	A/A	—	A	—	D
V-100	F	A/P.A.	0.4	A ₂	MN	CcDee
IV-15	F	A/A	0.4	O	MN	ccDee
V-16	F	A/A	0.3	O	N	CcDee
V-19	M	A/A	0.3	O	N	CCDee
V-43	M	A/A	0.3	O	MN	CcDee
V-45	M	A/P.A.	0.3	A ₂	N	ccDee
V-46	F	A/A	0.2	A ₂	MN	CCDee
V-71	F	A/A	0.3	O	MN	ccddee
V-72	F	A/A	—	A ₂	MN	ccddee
V-73	F	A/A	—	O	N	ccDE
V-74	F	A/A	0.3	A ₂	MN	ccddee
V-75	M	A/A	—	O	N	ccddee
V-76	F	A/A	0.3	A ₂	N	ccddee
V-77	M	A/A	0.3	A ₂	MN	ccddee
V-92	F	A/P.A.	0.4	O	MN	CcDee

"h" refers to the husband of the individual represented by the Roman numeral; "w" = wife.

*Grouped according to lines of direct descent for linkage detection.

Genetic Observations

Studies of 73 members of the proposita's family and of four other persons related through marriage (for linkage studies) yielded the results presented in table 2 and Figs. 3 and 4. Nineteen other persons in the kinship showed the same starch gel electrophoretic pattern as the proposita, *i.e.*, a small amount of the slow Hb P.A. component in addition to hemoglobin A and the "fast" Hb A₂-like substance. Five individuals in generation IV (Fig. 3) demonstrated this pattern and each transmitted it to his offspring. Four of the five have married normal persons; 18 of their offspring were examined, and of these, nine possessed electrophoretic findings comparable to those of the affected parent, both sexes being affected (table 3). This distribution, plus its presence in other members of the family who were not counted because of possible ascertainment bias, indicate that Hb P.A. is determined by a single autosomal gene.

One additional individual (IV-12) possessing hemoglobins A and P.A. married her first cousin, IV-11, himself the offspring of a first cousin mating (Fig. 3). His hemoglobin upon starch gel electrophoresis consisted mostly of Hb P.A. Of their eight offspring studied, five demonstrated predominantly the slow component, and three showed Hb A and Hb P.A. This distribution suggests that the father and five of the children are homozygous for the gene in question, the other three being heterozygous. The segregation data, therefore, reinforce the inference from the electrophoretic studies.

Blood group determinations were performed on all individuals under consideration in order to test possible linkage between the Hb P.A. gene and those responsible for the ABO, MN, and Rh systems.

Fig. 4 presents a portion of the pedigree, of Fig. 3, in which the blood group data are included. Information provided by this family rules out absolute linkage for the genes controlling Hb P.A. and those responsible for the ABO and Rh systems. Thus, in relation to the ABO locus, if we suppose that the *Hb P.A.*

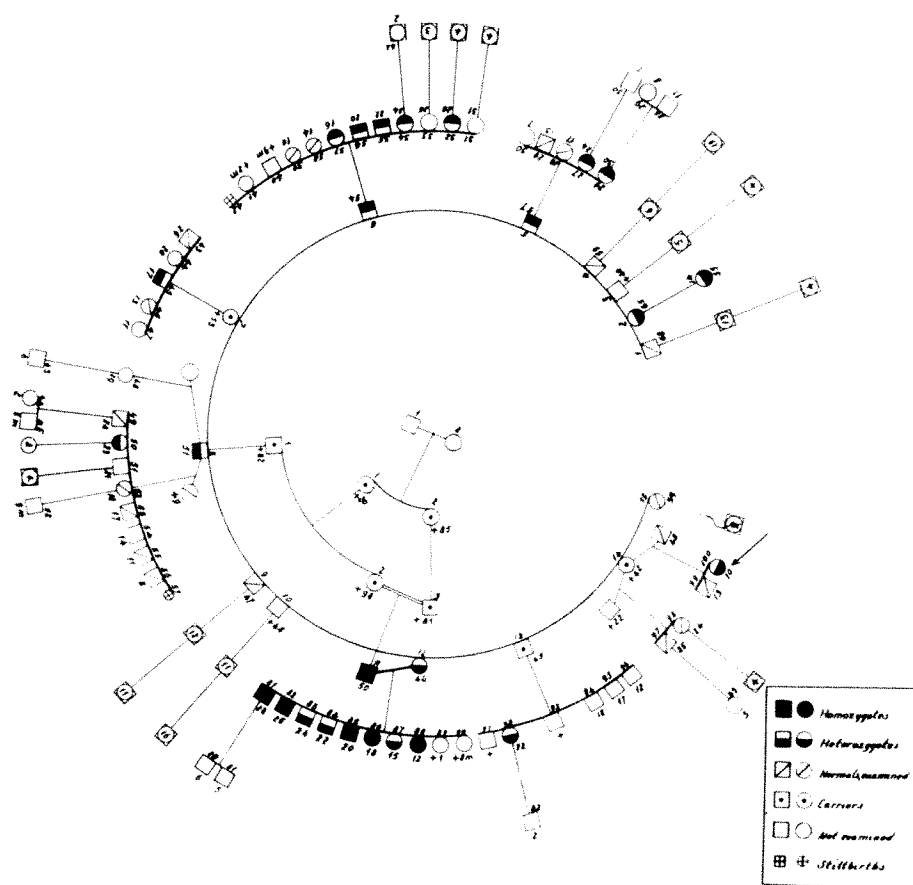


FIG. 3. Pedigree of family possessing hemoglobin Pôrto Alegre.

gene is on the same chromosome with I^{A_2} , all the O individuals in generation V should be of genotype $Hb A/Hb P.A.$ This is not the case: V-81 and V-88 have genotypes $Hb P.A./Hb P.A.$ Supposing that the gene is in the same chromosome as gene I^0 , then all O individuals should be $Hb P.A./Hb P.A.$, which again is not true. By similar reasoning it is possible to eliminate absolute linkage with genes from the Rh system. The sib-pair method of Penrose was applied to detect partial linkage, also with negative results. Linkage with the MN locus cannot be excluded.

The mutant gene responsible for hemoglobin Pôrto Alegre should be very rare, since we have not detected it in a random sample of 210 white (Tondo and Salzano, unpublished) and more than one thousand Negroid individuals (Tondo and Salzano, 1962 and unpublished) from the Pôrto Alegre population. No instance of it was observed in about 700 Indians and Mestizos from southern Brazil (Tondo and Salzano, 1960 and unpublished).

Further Characterization of $Hb P.A.$

On paper electrophoresis at pH 6.5, $Hb P.A.$ migrated with $Hb A$. On starch gel at pH 7.1 (Fig. 5) $Hb P.A.$ of the homozygote migrated very slightly

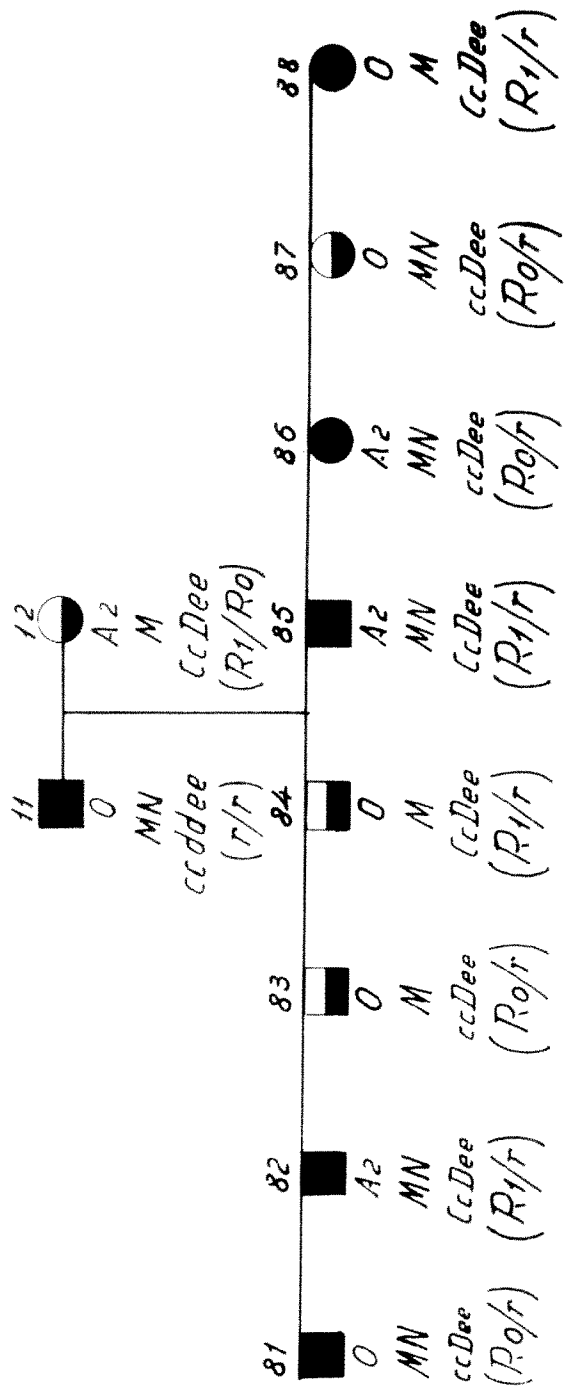


FIG. 4. Portion of pedigree showing linkage relationships of Hb P.A. and blood group antigens. Symbols same as in Fig. 3.

anodally to Hb A, not separating clearly from it. On agar gel electrophoresis at pH 6.5 (Fig. 6), Hb P.A. migrated with hemoglobin S for a short period of time. With more prolonged electrophoresis it apparently became denatured or lost its ionic charge and then moved more anodally, presumably by electro-

TABLE 3. OFFSPRING OF NORMAL BY Hb A PLUS Hb P.A. MATINGS

Male		Female	
Hb A 5	Hb A + Hb P.A. 2	Hb A 4	Hb A + Hb P.A. 7
pH 7.1			
A + C			
P.A.			
A + S			
A + E			
A + P.A.			

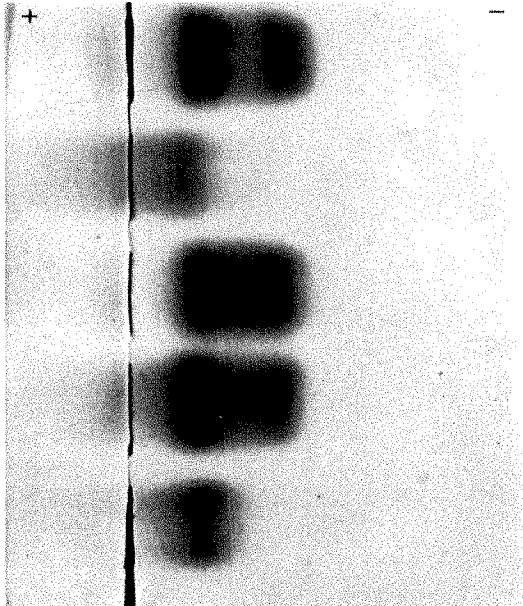


FIG. 5. Comparison of Hb P.A. with known hemoglobins on vertical starch gel electrophoresis in 0.1 M phosphate buffer, pH 7.1.

osmosis, until its position corresponded with that of Hb C. On that particular pattern the small amount of Hb A-like material of the homozygous Hb P.A. is not visible; Hb A₃ is probably included with the minor Hb F component.

An attempt was made to quantitate the Hb A₃-like substance by densitometry of the paper electrophoretic patterns. In the blood of five normal individuals, Hb A₃ constituted from 1.6 to 3.2 per cent of the hemoglobin (mean 2.8 per cent). In the five individuals possessing both Hb A and the slow component, the Hb A₃-like substance constituted from 3.9 to 8.1 per cent (mean 5.8) of the hemoglobin, and in those having predominantly the slow hemoglobins, the Hb A₃-like component varied between 7.5 and 15 per cent (mean 11.0).

Quantitation of the abnormal hemoglobin fraction on starch block electrophoresis was unsuccessful because Hb P.A. does not separate from Hb A at pH 8.6, giving the same pattern as on paper electrophoresis (Fig. 1). In six samples from heterozygotes the mean Hb A₂ concentration was 2.8 per cent by starch block electrophoresis, with values ranging from 2.3 to 3.3 per cent; the normal range for this laboratory is 2.5 to 3.5 per cent. In two homozygotes the Hb A₂ concentrations were 2.3 and 3.4 per cent.

The aforementioned initial starch gel electrophoresis and the family studies were performed on hemolysates prepared in Brazil and shipped to Ann Arbor;

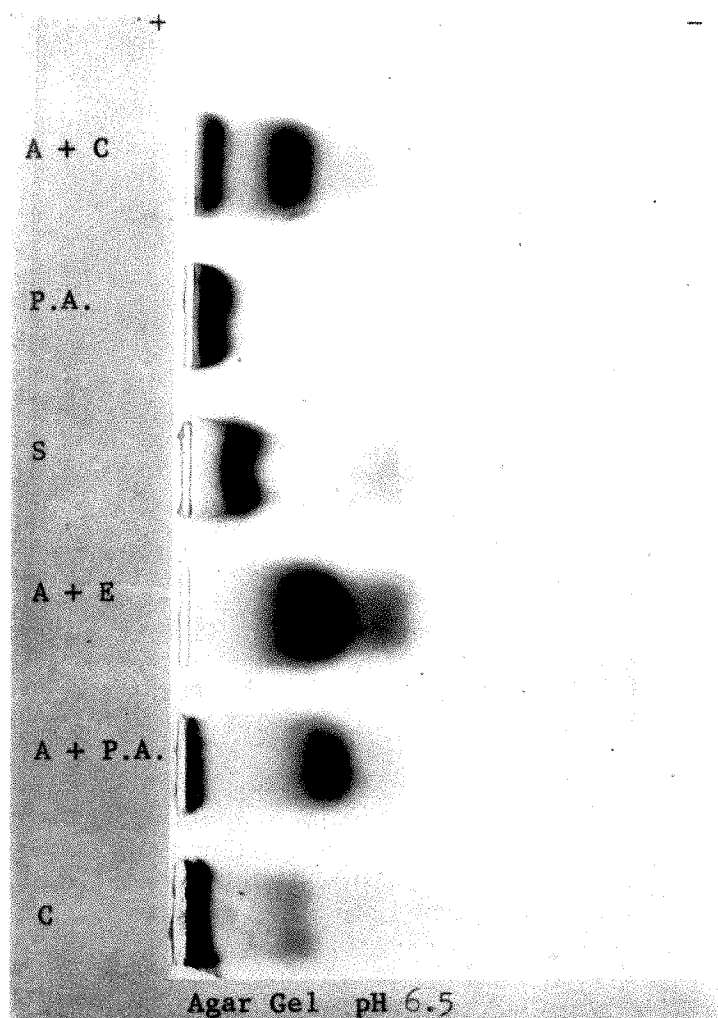


FIG. 6. Comparison of homozygote (P.A.) and heterozygote (A + P.A.) with known hemoglobins on agar gel electrophoresis in 0.03 M citrate buffer, pH 6.5. The minor rapidly migrating component in each sample is Hb F and Hb A₂.

the average age of the specimens at the time of electrophoresis was 6 weeks, at which point considerable methemoglobin was present in the hemolysates. Late in the study washed packed erythrocytes from two persons (V-82, a homozygote, and V-84, a heterozygote) were shipped to Ann Arbor in an attempt to repeat the studies on better preserved specimens. Upon preparation of the hemolysate by addition of distilled water and toluene and starch gel electrophoresis without prior freezing, both hemolysates showed the electrophoretic pattern of heterozygotes (Fig. 2a). However, 10 days later after storage at 4°C, as oxyhemoglobin, the electrophoretic pattern of V-82 was that of the homozygote, *i.e.*, predominantly the slowly migrating hemoglobin, whereas the minor amount of Hb P.A. in V-84 did not increase detectably (Fig. 2b). Further studies on other aliquots

of the fresh hemolysate indicated that oxidation with potassium ferricyanide facilitated this conversion to the homozygote pattern. Thus it appears that the development of the electrophoretic abnormality in the hemolysates of homozygous Hb P.A. individuals is a function of auto-oxidation of the abnormal molecule. Treatment of the hemolysate with sodium dithionite in the presence of carbon monoxide did not diminish the amount of the slow component, however.

The oxygenated ferrohemeoglobin form of Hb P.A. of a homozygote contained absorption maxima at 411.2, 536.2, and 572 $m\mu$, corresponding with those of normal hemoglobin. In three samples, reduction with sodium dithionite shifted two of the peaks to 427 and 552 $m\mu$, also normal. The maxima for carbonmonoxyhemeoglobin were found to be 415.5, 535, and 566 $m\mu$. The tryptophan notch at 290 $m\mu$ was similar to that of normal hemoglobin. The ratio of extinction coefficients at 290 and 536 $m\mu$ for Hb P.A. in the oxyhemeoglobin state is 1.80 compared with 1.90 for pooled hemolysates from three normal individuals. For carbonmonoxyhemeoglobin the corresponding ratio was 2.0. These findings indicate that the spectral characteristics of Hb P.A. are normal.

DISCUSSION

The distribution of Hb P.A. in this family in which matings of normal parents with individuals having a small amount of the abnormal hemoglobin produced offspring, half of whom showed a similar amount of the slow hemoglobin and half of whom were normal, suggests that Hb P.A. is inherited. The mating of an individual possessing predominantly Hb P.A. (IV-11) with a heterozygote (IV-12), producing five children with Hb P.A. as a major component and three having minor amounts of Hb P.A., further strengthens the evidence for simple mendelian genetic control of its production, despite the possibility that the original observation leading to its detection, *i.e.*, the increased amounts of Hb A₂, may be a result of augmented ageing effects and that its unique physicochemical properties may be the result of secondary alterations of the molecule produced in the preparation of the hemolysate. The existence of homozygotes greatly facilitated these investigations since laborious separation of Hb P.A. from Hb A was avoided.

Most of the large number of inherited variations of human hemoglobin described thus far have been characterized by variations in electrophoretic mobility (Rucknagel and Neel, 1961). It is now well established that these electrophoretic changes are the results of gene induced single amino acid substitutions into one of the two pairs of polypeptide chains of approximately 140 amino acid residues comprising the primary structure of the molecule. Thus the substitution of a positively charged amino acid for a negatively charged one at a specific residue of each of the two α - or β -polypeptide chains of the molecule will result in a net charge difference of $+4$ for the abnormal hemoglobin relative to Hb A.

Considering only the relative mobility of Hb P.A. on starch gel electrophoresis at pH 8.6, its position between hemoglobins S and C implies a net charge of $+2$ to $+4$ per molecule and necessitates differentiating it from hemoglobins E and O. The former possibility has been excluded by direct comparison on starch

gel at acid (Fig. 5) and alkaline pH and by agar gel electrophoresis (Fig. 6) and the latter by paper electrophoresis since Hb O migrates cathodally to Hb S at pH 8.6 (Lie-Injo and Sadono, 1958).

Heretofore, the relative mobilities of the abnormal hemoglobins have been similar on starch gel, starch block, and paper media at the same pH. The retarded movement of oxidized Hb P.A. on starch gel electrophoresis in contrast to more rapid mobility on the latter two is unique among the human hemoglobins, suggesting that it is either an abnormally shaped or unusually large molecule; in either case, its mobility is impeded by the small pore size of the starch gel. This phenomenon in itself is not unusual with other proteins. Indeed, a number of serum proteins, among them the haptoglobins, migrate relatively more slowly on starch gel than on paper, and these differences are due in large measure to variation in molecular weights (Smithies, 1959). That hemoglobin P.A. is a macromolecule larger than the normal one is also suggested by preliminary ultracentrifugal studies which show that it has a sedimentation velocity of 6 Svedburg units, compared with 4.1 S for normal hemoglobin and the hemoglobins of individuals having sickle cell trait. These observations will be presented in detail in a subsequent report which will define the structural alteration of the molecule more precisely. Thus the retarded mobility on starch gel electrophoresis compared with media having larger pore sizes appears not to be due to a strong positive charge, but rather to interaction of a larger hemoglobin molecule, perhaps an octamer, with the starch gel.

The normal electrophoretic mobility, even on starch gel electrophoresis, of most of the hemoglobin of the fresh unoxidized hemolysate of homozygous individuals, indicates that the gene determining Hb P.A. has not altered the net charge of the molecule, and that *in vivo* the molecule exists as the normal tetramer. This may well account for the lack of anemia, erythrocyte morphologic abnormalities, or associated clinical symptoms. Strictly speaking, then, one is justified in considering Hb P.A. as a "hidden mutant," as postulated by Neel (1959), inasmuch as examination of a freshly prepared hemolysate might contain neither the "slow" component nor the Hb A₂-like material. On the basis of the *in vitro* evidence for a macromolecule presented herein, one cannot exclude the possibility that the hemoglobin defect is secondary to an altered intracellular environment, such as presence of a polymerase, a high concentration of metal ions, or binding with a nonhemoglobin protein. The first possibility seems unlikely because of the marked difference between the amount of the macromolecular form in heterozygotes' and homozygotes' hemolysates and because of the conditions under which such polymerization has presumably taken place, and the last because of the large amount of additional protein required. The search for possible linkage relationship with blood group antigens might have given some indication of such alternatives, since linkage of the hemoglobin structural loci with blood group genes has not been conclusively demonstrated. The enhancement effect of potassium ferricyanide and freezing on the apparent polymerization of the molecule suggests that the abnormal molecule is susceptible to oxidation, perhaps even auto-oxidation, and that polymerization proceeds as a consequence. Inability to reverse the process with sodium dithionite which reduces the heme iron back to the ferrous state tends to eliminate the heme

group as a participant in the process and suggests that the globin moiety per se is oxidized.

The appearance of a large amount of the hemoglobin A₃ component cannot be overlooked since this led to the detection of Hb P.A. originally. In the normal individual, hemoglobin A₃ appears to increase with the ageing of the erythrocyte *in vivo* (Kunkel, 1958; Ranney and Kono, 1959). In a fresh hemolysate it constitutes approximately eight per cent of the hemoglobin on starch block electrophoresis and this is increased further upon storage *in vitro*. *In vitro* evidence indicates that hemoglobin A₃ may be formed by the complexing of glutathione with hemoglobin A (Huisman and Dozy, 1962), although the composition of naturally occurring Hb A₃ is somewhat in doubt. The increase in Hb A₃ in individuals with Hb P.A. suggests that the genetic alteration of the primary structure of the molecules has increased the glutathione binding capacity of the molecule, if this is the nature of Hb A₃. The delineation of the structural change in Hb P.A., therefore, may help define the site of attachment and the nature of the bond. The relationship between this effect of the Hb P.A. gene and the apparent ability of the molecule to polymerize under as yet poorly understood circumstances remains to be defined.

SUMMARY

A new inherited abnormal hemoglobin, designated hemoglobin Pôrto Alegre has been detected in a Caucasian Brazilian family. The anomaly was originally detected because of an increased amount of a hemoglobin A₃-like component on paper electrophoresis. On starch gel electrophoresis, however, a major or minor component, migrating between hemoglobins S and C, was observed in apparent homozygous and heterozygous individuals, respectively. The fraction migrated only very slightly slower than hemoglobin A on starch block electrophoresis. Other characteristics of Hb P.A. are described.

The variation in relative electrophoretic mobility on different zonal media, all at alkaline pH, suggests that Hb P.A. is a larger molecule than normal, possibly an octamer. The sedimentation velocities of 6 S for Hb P.A. compared with 4 S for Hb A strengthen this hypothesis. The observation that in fresh hemolysates from homozygotes' Hb P.A. there is a minor component which increases in amount upon oxidation, freezing, and storage suggests that the result of the mutant gene is an alteration in the hemoglobin molecule which makes further polymerization possible, and that in a fresh hemolysate Hb P.A. is nearly a "hidden mutant." Linkage with two common blood group antigens has been excluded.

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Serum Cholinesterase Levels in Families and Twins

NANCY E. SIMPSON¹ AND W. KALOW

Department of Pharmacology, University of Toronto, Toronto, Canada

THE ACTIVITY OF pseudocholinesterase in human plasma has long been known to vary substantially from person to person (Wolfsie and Winter, 1952; Augustinsson, 1955). In some individuals, low pseudocholinesterase activity can be accounted for by hereditary alterations of the properties of the enzyme. In this paper the terms cholinesterase and esterase shall be used when referring to this enzyme.

Two alleles control esterase types which have been termed "usual" (normal) and "unusual" (atypical) esterases and are distinguished by dibucaine inhibition (Kalow, 1962; Kalow and Staron, 1957; Harris, Whittaker, Lehmann and Silk, 1960). A third type of esterase (distinguished by sodium fluoride inhibition, Harris and Whittaker, 1961) is controlled by a gene which may be an allele of those controlling the first two types (Harris and Whittaker, 1962). Liddell, Lehmann and Silk, (1962) have supplied evidence for the existence of yet another gene which they have named "silent" and which causes lack of esterase activity. Harris, Hopkinson and Robson (1962) have described a genetically determined variant of cholinesterase distinguished by 2 dimensional electrophoresis. The genes causing "atypical" esterase, unusual fluoride inhibition, lack of esterase activity and the variant determined by electrophoresis are all relatively rare. The great majority of persons seem to be homozygous for the gene producing the normal type of pseudocholinesterase. The present investigation is exclusively concerned with this most common type of enzyme.

Even among healthy persons with the normal type of pseudocholinesterase, the activity of this enzyme varies greatly (Fig. 1), although for any given individual esterase levels remain fairly constant (Augustinsson, 1955; Lindsay, 1955). A part of this variation may be accounted for by influences of age and sex (Kalow and Gunn, 1959). If one corrects for these factors, there still remains a large person to person variation of cholinesterase levels. This paper describes our attempts to determine whether this variation of enzyme level is due to hereditary or environmental causes.

METHODS

The present series of tests consisted of two parts. First, serum cholinesterase levels were investigated in 107 persons of 25 different families. Persons heterozygous for atypical esterase occurred in four additionally investigated families, but all data on these families were excluded so that the present report deals only with the normal type of esterase.

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¹Queen Elizabeth II Scientist.

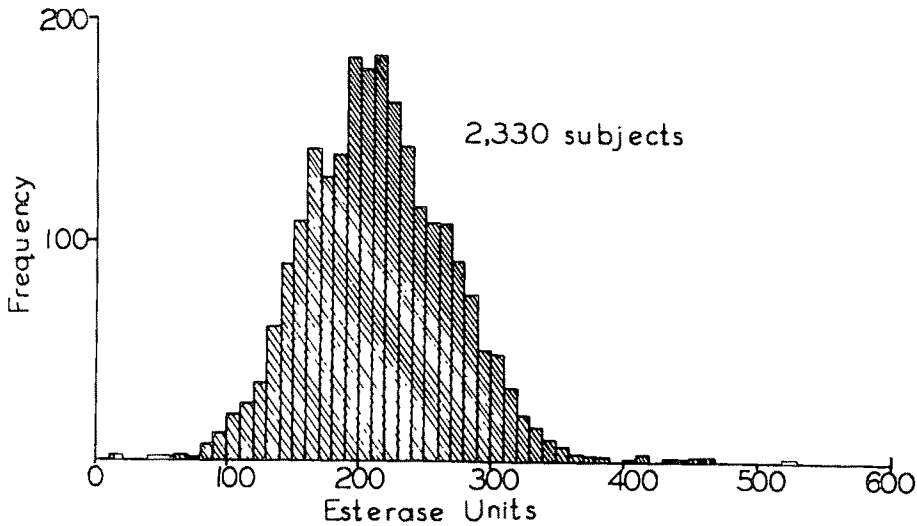


FIG. 1. Range of variation of cholinesterase activity in a normal population. The sampled population is essentially that described as "healthy Canadian population" by Kalow and Gunn (1959). The additional 300 samples were obtained as outlined in that paper. Thus, the great majority of individuals were white adults. They were not known to be sick, and their esterase type was normal as judged by dibucaine inhibition. The distribution curve is slightly, but significantly, skew and skewness cannot be eliminated by using the logarithms of units.

As a second and separate study, esterase levels of 28 pairs of twins were determined. The twins ranged in age from 3 to 26 years. Fifteen pairs were monozygotic (MZ), the other 13 were dizygotic (DZ) twins. The zygosity of the like-sexed twins was determined by the following criteria. For most of the twins, the fetal membranes of the placentae had been previously examined (by Dr. Irene Uchida, then of the Department of Genetics, Hospital for Sick Children, Toronto) and classified as monochorionic or dichorionic. For all pairs of twins, and where possible for their parents, eight blood groups were determined (by Dr. Bruce Chown, Rh Laboratory, Winnipeg), the dermatoglyphics were analyzed for similarity and serum cholinesterase was qualitatively classified.

Blood samples were obtained by venipuncture; the serum was removed as soon as possible and stored in a frozen state. A factor which may enhance esterase concentration in a blood sample is venous congestion while taking the blood. A tight cuff around the arm causes loss of plasma water into tissues. The importance of this factor for determinations of hematocrit has long been recognized (Landis, Jonas, Angevine and Erb, 1932). We came to appreciate this factor for esterase determinations only after the sampling for this study had been completed. Although some variation of esterase level is introduced in this way, it probably does not critically affect conclusions made from the data. Esterase determinations were performed by ultraviolet spectrophotometry, as previously described (Kalow and Lindsay, 1955). The method consists in measuring the hydrolysis of benzoylcholine by observing a spectral shift at 240 mμ. Sources and magnitudes of experimental errors have been discussed (Kalow and Genest, 1957). To overcome day-to-day variation, the activity of a

purified standard preparation was determined daily, and the experimental data adjusted accordingly. In contrast to the practice of some previous reports, the units of esterase activity used in this paper indicate shift of absorbance during three minutes, multiplied by 100. If these units are multiplied by the empirical factor 1.18 they give micromols of acetylcholine hydrolyzed by 1 ml. of serum at 37°C during one hour. The type of esterase was assessed (Kalow and Genest, 1957) by determining the dibucaine number (DN), *i.e.*, enzyme inhibition with dibucaine under standardized conditions. All utilized samples had a DN above 76. This selection probably eliminated individuals with unusual sodium fluoride numbers. The determination of fluoride numbers (FN) as a criterion for esterase abnormality was unknown when this investigation was started and could thus not be tested for all subjects. However, decreased FN are, as a rule, accompanied by slightly decreased DN (Harris and Whittaker, 1961). It is thus very likely that no persons with lowered FN occurred in this series.

RESULTS

Family Data

When all members of the 25 families were considered as a single population, the esterase levels showed about the same variation (table 1) as has been described in other populations (Augustinsson, 1955; Kalow and Gunn, 1959).

TABLE 1. VARIATION OF SERUM CHOLINESTERASE ACTIVITY
IN 25 FAMILIES

Family Data	No. of Persons	Mean Esterase Level	Standard Deviation
All persons	107	201.7	42.5
Fathers	25	210.3	32.7
Mothers	25	178.8	33.6
Adults	57*	187.5	35.7
Children	50	217.8	44.5

*Seven offspring who were over 19 years old are included in this group.

The family data did not show any pattern of esterase levels which could be taken to suggest Mendelian segregation. However, the mean esterase level of each pair of parents was significantly correlated with the mean esterase level of their children ($r=0.45$; $p=0.01$). Correlations of esterase levels between fathers and children, and between mothers and children were each significant ($p<0.05$). The variance of esterase levels between families was greater than within families ($p<0.01$) (table 2). Among adults, esterase levels are slightly

TABLE 2. ANALYSIS OF VARIANCE FOR FAMILY DATA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Between families	24	71,913.9	2,996.4	
Within families	82	101,355.8	1,236.0	2.42*
Total	106	173,269.7		

All data were corrected for sex or age (see text)

* $p<0.01$

correlated with body weight (Kalow and Gunn, 1959), and presumably more closely with body fat (Berry, Cowin and Davies, 1954). However, no attempt was made in the present study to correct esterase levels for body weight. First, there was no significant correlation between mean body weights and mean esterase levels of the parents, and secondly, it would have been difficult to distinguish among the children between effects of body weight because of differences in age and stature.

Familial tendency may be caused by common environmental factors or by heredity. These two can be distinguished sometimes by comparing intraclass correlations for pairs of parents and that for sibships. However, before attempting to assess these variations, it was thought to be appropriate to adjust the data for the secondary effects of sex and age.

Mean esterase levels were significantly different for parents and children ($p < 0.01$), apparently due to the influence of age. The levels dropped by 2.52 ± 1.18 units per year in the age group from 3 to 19 years ($r = -0.286$, $p < 0.05$). The mean age of children was 9.1 years. The influence of age among the adults was not significant in the present sample (*cf.*, however, Kalow and Gunn, 1957, 1959). On the other hand, adult esterase levels were higher in males than in females ($p < 0.05$; table 1), but this sex difference was not significant among the children in the present sample. Hence the data on offspring were adjusted for age while the data on parents were corrected for the influence of sex. Children's levels were expressed for 10 years of age, using the equation, adjusted level = observed level $- [2.5 \times (10 - \text{age in years})]$. For example, the adjusted level of a child aged 14 years having an observed level of 200 would be $200 - [2.5 \times (10 - 14)] = 210$. Fifty observations were thus adjusted. One value of a child below 3 years of age was omitted, and the observed values of seven offspring above the age of 19 were left unadjusted. In order to adjust for the sex influence upon the esterase activity of adults, the levels of all mothers were increased by a constant factor. The equation was, adjusted level = observed level $\times (\text{mean level of fathers} / \text{mean level of mothers})$. For example, a mother with observed units of 200 had an adjusted level of $200 \times (210.3/178.8) = 236$.

Intraclass correlations (Snedecor, 1956) for the corrected data are given in table 3. The correlation within the sibships (0.42) is not significantly greater than within their parent pairs (0.30). This observation does not provide evidence for genetical control of esterase levels, although multigenic control could exist and not be detected in this small sample. It should be noted that the differences between families were due to greater variation between sibships rather than between pairs of parents.

Twin Data

An independent means of evaluating hereditary and non-hereditary factors is given by a comparison of fraternal (DZ) and identical (MZ) twins. In respect to any purely hereditary trait, identical twins (who have identical genes) should be as one person and fraternal twins as different as ordinary sibs. In respect to purely environmental influence, identical twins should differ as much from each other as do fraternal twins. Usually, however, there is a contribution of both heredity and environment towards a given trait. Kemp-

TABLE 3. INTRACLAS CORRELATIONS FOR PARENTS, F₁ SIBSHIPS AND TWINS

	Pairs of Parents*	F ₁ Sibships	MZ Twins	DZ Twins	MZ Male Twins	MZ Female Twins
B	1185.5	2922.4	1163.0	3243.8	173.0	2508.9
W	645.7	1115.4	219.5	554.6	87.5	372.4
σ_x^2	915.6	1889.7	691.3	1899.2	130.6	1440.6
r_1	0.30±0.18	0.42†	0.68±0.04	0.71±0.04	0.33±0.09	0.74±0.05
No. of Individuals	50	57	30	26	16	14

B = mean square for between groups.

W = mean square for within groups.

 σ_x^2 = variance for individuals. r_1 = intraclass correlation and standard deviation (very approximate).* = parent pairs refer to parents of F₁ sibships, but not to parents of twins.

† = data are not suitable for calculation of standard deviation.

thorne and Osborne (1961) pointed out that if the two types of twins differ mainly in respect to genetic constitution for the factor in question, the variance for individuals should be the same for the two types of twins, but the intraclass correlation for MZ pairs should be greater than that for DZ twin pairs.

Intraclass correlations for cholinesterase levels in 15 pairs of MZ and 13 pairs of DZ twins are given in table 3. Since most of these twins were young, their levels were corrected for age as described for offspring in the family data. Although numbers are small, the between pair differences for cholinesterase levels indicate a familial tendency as found in the family data. The intraclass correlations, however, are essentially the same for the two types of twins, which suggests that environmental rather than hereditary influences are implicated. It is of interest to note that the intraclass correlation for male MZ twins is significantly less than that for female MZ twins.

Comparison of the intraclass correlations for twins should be accepted with reservation. The individual variation and that between pairs of MZ twins for some unexplained reason is less than that for DZ twins. The intraclass correlation for DZ twins appears to be greater than that within sibships from the family data, although these two groups are from different families. The over all comparison of intraclass correlations for MZ and DZ twins, however, suggests environmental rather than hereditary influence.

DISCUSSION

It is reasonable to suppose that individual differences in rate of formation or rate of destruction of the normal enzyme account for the considerable variation of its activity. If either of these rates were controlled by a single gene, evidence for hereditary control of normal enzyme levels could be expected. Such evidence, however, has not been found. If both rate of formation and rate of destruction were controlled by independent genes, a given person would have at least 2 pairs or 4 genes, responsible for esterase levels. Such multigenic control might be difficult to assess in family data, but should show up in twin data. Since this was not the case, it appears that environmental factors may control the balance of formation and destruction of plasma cholinesterase.

Genetic control of low activity of the normal type of esterase was suggested by observations in one family (Kalow, 1959). It was postulated that some individuals with low activity exhibited the effect of one normal gene and one gene for no activity. This hypothesis has been supported recently by observations of Liddell *et al.* (1962) who used the term "silent" gene. This gene cannot be identified unless it occurs in the homozygous state or in combination with the atypical gene, and unless the esterase types of relevant family members are known. It appears likely that this gene was not encountered in the present study, and is probably rare.

The blood taken for cholinesterase determinations was usually taken from all members of a family and twin pairs at the same time. Wetstone, Tennant and White (1957) have stated that the mean cholinesterase activity in fasting individuals is less than that in postprandial individuals. Although time in relation to the last meal was not taken into consideration when the blood samples

were collected in the present study, this would be constant within families and twins, but would vary from family to family and from twin pair to twin pair. This factor could contribute in part to the finding of larger differences between families and twin pairs than those occurring within families and twins. However, investigations still in progress suggest that this factor, if it does exist, is of minor importance and cannot account for the similarities of esterase levels within families. This does not exclude the possibility of long-range influences of enzyme suppressors or inducers that might be contained in specific foods habitually ingested by families or individuals. Cholinesterase formation could be induced by Goldstein (1959) in *Pseudomonas fluorescens*. The most effective inducer was choline which is not a substrate; esters of choline seemed to induce only by virtue of supplying choline on hydrolysis. Glucose prevented the induction. Burkhalter, Featherstone, Schueler and Jones (1957) presented evidence which suggests that choline or cholinesters are able to induce cholinesterase formation in explanted chick embryos. Remmer (1962) has found it possible to induce esterase (presumably pseudocholinesterase) formation with the aid of phenobarbital in the liver of young rats.

SUMMARY

Serum cholinesterase levels were determined in 107 persons from 25 families and in 15 pairs of monozygotic twins and 13 pairs of like-sexed dizygotic twins. All of these persons appeared to have the normal type of esterase. Four additional families in which individuals were heterozygous for atypical cholinesterase were excluded from the study. Analysis of variance with intraclass correlations for parents, sibships and twin pairs suggest that the observed variation in activity levels are largely environmentally determined, and no evidence for important hemogenic or multigenic control of cholinesterase levels was found.

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ADDENDUM

It has just been published (Harris, Hopkinson, Robson, and Whittaker. 1963. Genetical studies on a new variant of serum cholinesterase detected by electrophoresis. *Ann. Hum. Genet.* 26: 359-382) that the sera of individuals who have the variant of cholinesterase distinguished by two dimensional electrophoresis (the C_s variant) have a 30 per cent higher mean level of activity than sera from phenotypically normal individuals without the C_s variant. This is in contrast to the low activity of sera from individuals who have the 'silent' gene. The sera of 5 per cent of unrelated British individuals had the genetically determined C_s variant. It is therefore possible that a few families and/or twins with the C_s variant were included in the above study but probably not frequently enough to alter the general conclusion.

Polydactyly of the Second Metatarsal with Associated Defects of the Feet: A New, Simply Inherited Skeletal Deformity

JAMES V. NEEL AND MALCOLM L. RUSK¹

*Department of Human Genetics, University of Michigan
Medical School, Ann Arbor, Michigan*

THIS NOTE WILL REPORT a skeletal anomaly of the feet which appears in three members of two generations of a kindred. The common pathological feature is a type of polydactyly whose exact counterpart does not seem to have been described previously.

CLINICAL MATERIAL

Five members of the kindred have been examined in the Heredity Clinic. III-1 (Fig. 3), the 38 year old propositus, was referred to the Clinic for genetic counseling. He complained of pain in his feet on weight-bearing, sufficient for him to plan elective surgical correction of the deformity. His health was otherwise good. On physical examination the major features in the feet (Fig. 1)



FIG. 1. Photograph of the feet of the propositus, III-1.

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¹Present address: Department of Medicine, School of Medicine, University of California Medical Center, Los Angeles, California.

were a bilateral dorsal eminence in the region of the second metatarsal, shortening of the left fifth metatarsal, hallux valgus, general equal forward projection of the second, third, and fourth toes, and overlapping toes. X-rays (Fig. 2) revealed an incomplete division of the second metatarsal bilaterally, with the

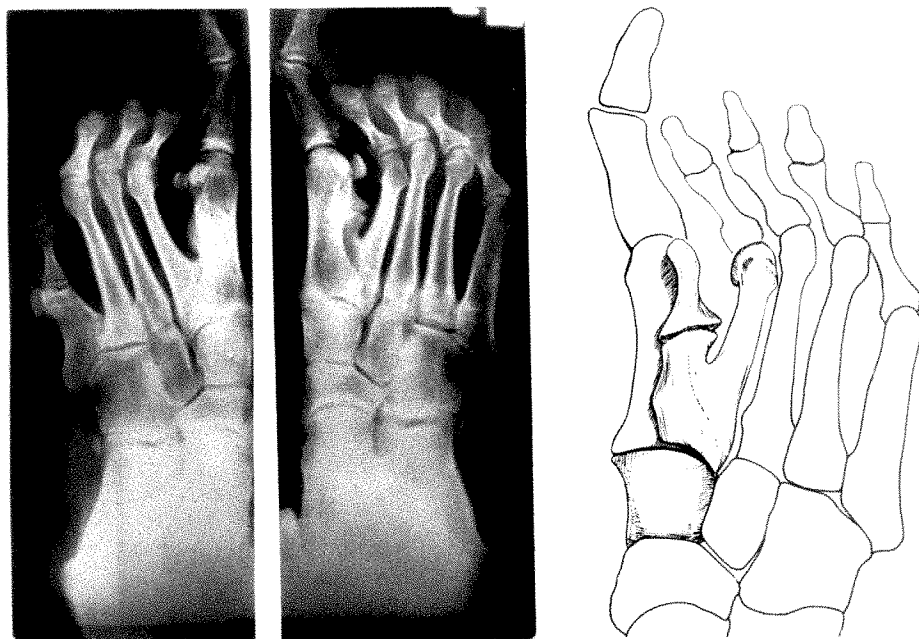


Fig. 2. The x-ray findings and their interpretation in the case of the propositus, III-1.

resulting "spur" capped by a single "phalanx." (The additional "phalanx" which appears in the x-ray is actually the sesamoid bone regularly present at the metatarsophalangeal joint of the great toe.) In addition, there was relative broadening and elongation of the first metatarsal and phalanges, and marked shortening and widening of the fifth metatarsal on the left. The first and second cuneiform bones appeared fused. There were no other obvious skeletal abnormalities, but complete x-ray studies were not performed.

The pedigree of the kindred obtained from the propositus constitutes Fig. 3. Two additional affected family members were seen. III-6, a 17 year old brother of the propositus, had no complaints about his feet. Photographs of his feet are reproduced in Fig. 4. The same general features, perhaps even more marked, are present in his feet as in those of the propositus. IV-4, the propositus' 13 year old son, had no complaints but had abnormal feet. On physical examination, the great toes projected disproportionately far forward. X-rays of the feet showed small bilateral exostoses from the proximal superior medial aspect of the second metatarsals. These exostoses resemble those seen in the propositus' feet in point of origin and orientation but differ in being much smaller.

Two other children of the propositus, IV-1, a 17 year old daughter, and IV-2, a 15 year old son, were examined and found to have normal feet. Two unexamined members of the kindred, as indicated in the pedigree, were

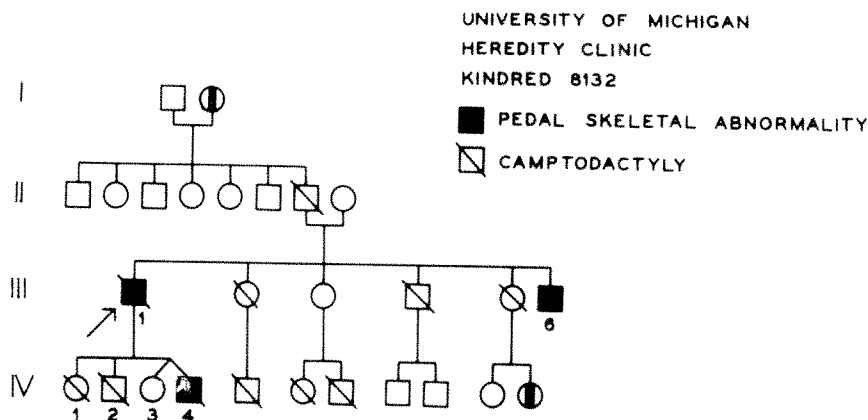


FIG. 3. Pedigree of a family showing an unusual form of polydactyly.

reported to have abnormal feet, but it proved impossible to obtain more detailed information. The propositus is the offspring of a marriage between third cousins. Camptodactyly (permanent flexion of the little finger) is also reported in the kindred; the affected are indicated in the pedigree as the propositus reported them to be. In the members examined, the camptodactyly was mild, in that the fifth fingers were curved but not stiff. Since this is a trait subject to overdiag-



FIG. 4. Photograph of the feet of III-6, the 17 year old brother of the propositus.

nosis by the layman, we accept the reported distribution with reservations, and regard its occurrence in this family as unrelated to the polydactyly.

DISCUSSION

So far as we have been able to determine, the precise counterpart of this type of polydactyly, involving a bifid second metatarsal bone, has not been previously described. The closest approximation appears to be a bilaterally bifid fifth metatarsal described, without family history, by Dommissé (1955). In the parental generation of the family here described, the accessory spur is large, with an abortive phalanx at its tip. In the propositus' younger son, the spur is small and has no surmounting phalanx. A variety of other deformities are found in the feet of the affected members: namely, short thick metatarsals, especially the fifth; fusion of the first and second cuneiform bones; hallux valgus and overlapping toes. The condition appears most likely due to a single dominant gene of varying penetrance and expressivity. In this connection, it is clear that had the abnormalities in the affected son of the propositus been slightly less, he might have passed for normal on physical examination, *i.e.*, among the reportedly normal transmitters of the gene x-rays might reveal penetrance where physical examination did not.

In recent years it has become fashionable in the field of blood group genetics to refer to "private" factors, known only in one or a very few families. The present family helps remind us that other components of the body may provide equally good examples of "private" genes.

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Evidence for the Absence of Detectable Linkage Between the Genes for Duchenne Muscular Dystrophy and the Xg Blood Group

JAMES I. CLARK, ROBERT H. PUIITE, RAYMUND MARCZYNSKI
AND JOSEPH D. MANN

*Butterworth Hospital and the Muscular Dystrophy Clinic
of Blodgett Memorial Hospital, Grand Rapids, Michigan*

SHORTLY AFTER THE Xg^a blood group antigen was discovered, we reported briefly that the locus on the X chromosome for the gene for this antigen was not close to that for muscular dystrophy of the Duchenne type (Mann *et al.*, 1962; Clark *et al.*, 1962). This conclusion was forced upon us by the seemingly independent segregation of the two genes in the families of patients with pseudo-hypertrophic progressive muscular dystrophy. The formal analysis of these data, with the aid of a computer, has confirmed the high estimate of the recombination fraction.

MATERIAL AND METHODS

Collection of Pedigrees

The case records of the Muscular Dystrophy Clinic of Blodgett Memorial Hospital were reviewed. All living cases of characteristic childhood muscular dystrophy were surveyed for possible study. In all, 53 pedigrees were assembled. All the families lived in Michigan (exclusive of Detroit) or in northern Indiana. Most of the cases came from around Saginaw, Grand Rapids and Muskegon, in that order of frequency. The families were of varied ethnic origin, but all but one were of Caucasian extraction. The exception was a Negro family with two typically affected sons (Pedigree No. 25).

The cases all presented the classic pattern of Duchenne muscular dystrophy. The onset varied somewhat from family to family, but most affected individuals showed early signs around age two, and all showed unequivocal involvement by age 6. At the time they were studied, most of the patients were confined to wheelchairs and some were bedfast. A few patients were in their early 20's but the oldest patient in the series was only 25.

Families having boys between the ages of 2 and 15 were studied clinically to be as sure as possible that no affected children were overlooked. In a few instances serum aldolase determinations were made to lend additional support to the clinical judgment that a given boy was not affected.

Selection of Suitable Pedigrees

Testing for Xg^a was begun at the same time that the pedigrees were being

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assembled and some families later proved unsuitable. In three of the rejected pedigrees, the dystrophy did not follow an X-linked pattern. Other families had only one affected son. We considered that such individuals might well be mutants and that the mother was, therefore, not necessarily heterozygous for the gene for muscular dystrophy. Two pedigrees were uninformative for linkage because the mothers were Xg(a-) and so, of course, were all the sons.

Usable Pedigrees

Seventeen pedigrees showed clear evidence of maternal heterozygosity for muscular dystrophy. Seven of these mothers also were demonstrably heterozygous for Xg^a. Six proved their heterozygosity by producing Xg(a-) sons; one (Pedigree No. 8) had an Xg(a-) father whose paternity could not be disproved by the evidence of multiple blood group antigens. The seven proved doubly heterozygous women provided the data for our preliminary report (1962) (Data in table 1). All 17 families were included in the computer analysis. The data on the mothers, proved heterozygous for muscular dystrophy but not for Xg^a, are included in table 2. The gene frequency $Xg^a = 0.651$, as calculated from the data of Sanger *et al.* (1962), was used in this portion of the analysis.

Immunohematology

The immunological testing in this study employed conventional methods. Red cells were collected by venipuncture except from a few infants from whom only capillary blood could be obtained. Testing for the Xg^a antigen was done in duplicate with the usual indirect antiglobulin method. In a few instances where weak or uncertain reactions were obtained, additional testing and absorption and elution studies were performed.

The ABO antigens were routinely determined in this series, and other blood groups, including Rh, Kell, Duffy, and MN systems, were done occasionally. Since we were concerned principally with mothers and sons, the paternity of the affected children did not influence our data. Indeed, a number of our patients were known to be half-brothers. When the paternity of the mother was of interest (*e.g.*, Pedigree No. 8), more elaborate testing was carried out.

RESULTS AND LINKAGE ANALYSIS

The 17 pedigrees contained 53 sons. Twenty-one sons were produced by the seven women proved doubly heterozygous.

The lod results for the muscular dystrophy x Xg linkage data are given in table 3, for a range of recombination values, θ . Since, in this full analysis, no selection occurred at one of the loci, Xg, no ascertainment correction is needed.

The maximum likelihood estimate of θ is clearly 0.5 but to derive a useful lower confidence limit, it is necessary to take into account the prior probability favoring loose, rather than close, linkage on a longish chromosome such as the X. A formula for calculating such a prior distribution, $f(\theta)$, is given on page 282 of Morton (1955) provided an estimate can be made of the length of the X chromosome in terms of map units. Such an estimate can be made, rather crudely, by making the approximation that map length is proportional to

TABLE 1. MOTHERS DOUBLY HETEROZYGOUS FOR MUSCULAR DYSTROPHY AND X_g^a

Pedigree No.	Mother's X _g a Reaction	Her Sons		Her Spouse(s)	Her Daughters		Her Father's X _g a Reaction	Her Family History For MD	Remarks
		X _g (a+)X _g (a-) MD+	X _g (a+)X _g (a-) MD-		X _g (a+)X _g (a-) MD+	X _g (a+)X _g (a-) MD-			
1	Dead	1	1	1	1		Unknown	None	
4	+	2	1	2			—	None	
5	+	2		1	1		Unknown	None	
8	+	3		Not tested			—	Pos.	Four affected uncles
25	+	1	1	Not tested			Unknown	None	One normal son not tested
35	+	1	1	1	2		Unknown	None	Also one X _g (a+) infant son of uncertain MD status
37	+	1	1	Not tested	2	1	Unknown	Pos.	One affected uncle; one affected cousin

Data previously published in part (Clark *et al.*, 1962)TABLE 2. MOTHERS PROVED HETEROZYGOUS FOR MUSCULAR DYSTROPHY BUT NOT FOR X_g^a

Pedigree No.	Mother's X _g a Reaction	Her Sons		Her Spouse(s)	Her Daughters		Her Father's X _g a Reaction	Her Family History For MD	Remarks
		X _g (a+)X _g (a-) MD+	X _g (a+)X _g (a-) MD-		X _g (a+)X _g (a-) MD+	X _g (a+)X _g (a-) MD-			
2	+	4			1	5	+	None	
3	+	1	2	1		1	+	None	
6	+	1			1		Unknown	None	1 MD son dead.
10	+	3		1		1	Unknown	None	1 Affected nephew
12A	+	1		1			+	Pos.	12A's maternal grandmother was 12B's great grandmother
12B	+	1	4	Not tested			Unknown	Pos.	
15	+		3	1			Unknown	None	
18	+	1	2	Not tested	3		Unknown	None	
27	+		2	1			Unknown	None	
28	+	1	1	1	2		Unknown	None	1 MD son dead
36	+	1	1	1	3		+	Pos.	} Sisters
36B	+	1	1	1	2		+	Pos.	

apparent length at metaphase and by allocating to the X chromosome an appropriate fraction of the total 3,000 map units which is slightly modified from the estimate (2,790 units) made by Ford and Hamerton (1956) for the total human autosomal complement. From table 41 of Maynard-Smith, Penrose and Smith (1961), we find that the X chromosome is equal to 0.0584 of the length of the total autosomal haploid set at metaphase. Thus the X might be roughly $0.0584 \times 3,000 = 175$ units long (*i.e.*, 1.75 Morgans). The curve for $f(\theta)$, when $L = 1.75$ Morgans, is of the same general shape as that given in Fig. 1 of Morton (1955) for $L = 1.5$ Morgans but rises to a higher maximum, 16.55 at $\theta = 0.4975$. It is convenient to reduce this maximum to 1 (and to take it as occurring when $\theta = 0.5$) by dividing all prior probabilities by 16.55. The \log_{10} of the resulting relative probabilities are then in the same form as the ordinary lod's and might be termed "a priori lod's." The two sets of lod's, when added, give the final lod's and the antilogs of these can then be plotted against θ . These antilogs represent the relative probabilities of various values of θ and are

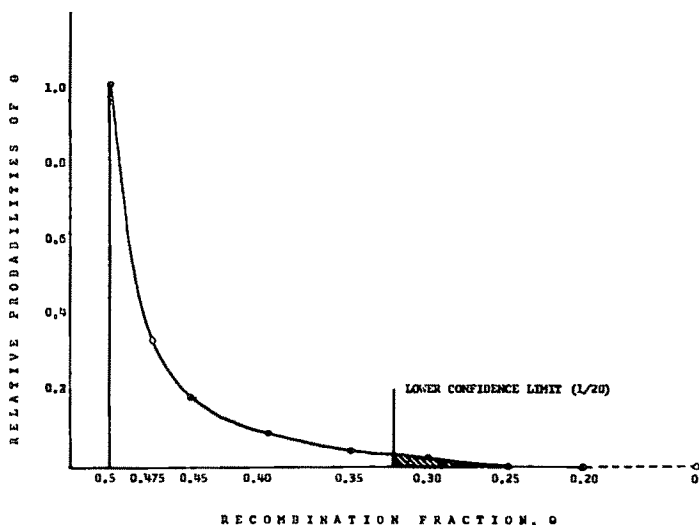


FIG. 1. Relative probabilities for the recombination fraction θ between the loci for the Xg blood group and Duchenne muscular dystrophy.

plotted in Fig. 1. The lower confidence limit (1/20) can be found by determining what value of θ divides the area under the curve in the ratio 19:1. By this method, based on the approach of Smith (1959), it is found that there is only one chance in 20 that the recombination fraction is lower than 0.33. It might be noted that the fair proportion of X-borne pairs of loci showing high recombination values is, so far as it goes, in general agreement with the above estimate of the map length of the X.

FINAL COMMENT AND SUMMARY

It is a disappointment that such an important gene as that for Duchenne muscular dystrophy should lie so far from the Xg locus, but at least the evidence

TABLE 3. LOD RESULTS FOR MUSCULAR DYSTROPHY x X_G LINKAGE DATA FOR A RANGE OF RECOMBINATION VALUES, θ .

	$\theta =$	0.5	0.475	0.45	0.40	0.35	0.30	0.25	0.20	0.10	0.05	0
a. Lods (data)		0	-0.017	-0.046	-0.147	-0.313	-0.566	-0.935	-1.475	-3.577	-6.036	$-\infty$
b. Tentative prior lods		0	-0.472	-0.677	-0.881	-0.992	-1.063	-1.110	-1.141	-1.169	-1.170	-1.161
c. Tentative final lods		0	-0.489	-0.723	-1.028	-1.305	-1.629	-2.045	-2.616	-4.746	-7.206	$-\infty$
Antilog (c) = relative probabilities of θ		1	0.325	0.189	0.094	0.050	1.023	0.009	0.002	2×10^{-5}	6×10^{-8}	0

Maximum likelihood estimate of $\theta = 0.5$.Lower confidence limit (1/20) = 0.32
(obtained graphically from curve of final probabilities, truncated at $\theta = 0.5$).

for the wide separation is convincing. The recombination fraction is estimated as 0.50; its lower limit ($1/20$) is 0.32, if certain prior probabilities are taken into account. One would suppose that the two loci either are at opposite ends of the same arm of the X chromosome, or else are on opposite arms.

ACKNOWLEDGMENTS

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1. The Concept of Genetic Load: A Critique

L. D. SANGHVI²

*Department of Human Genetics, University of Michigan
Medical School, Ann Arbor, Michigan*

CURRENT METHODS of assessment of genetic hazards of radiation in man depend on answering satisfactorily three broad questions. The first question requires information on the incidence of diseases, defects, and deaths primarily of genetic origin, an estimate of our hereditary burden. The second question relates to an ascertainment of the fraction of this burden that is dependent for its maintenance on recurrent mutation. The third question is concerned with determination of relationships between a given dose of radiation and a corresponding increase in mutation rates under a variable set of physical and biological conditions. The available evidence from observations in man and experimental organisms is largely restricted to answering the first and the third questions. The second question has given rise to theoretical thinking, some speculation and some controversy. (United Nations, 1962).

If our hereditary burden is maintained by recurrent mutation, any permanent increase in current radiation levels would proportionately increase such ill-effects eventually. Muller (1950) presented a most illuminating thesis along this line. An alternative mechanism has long been known to students of population genetics which could maintain, in theory, a major fraction of this burden without necessarily relying on recurrent mutation (Fisher, 1930; Wright, 1931; Haldane, 1932; Ford, 1940). Under the simplest genetic model of a population, with segregation of two allelic genes *A* and *a* at a locus, this mechanism depends upon a selective advantage of the intermediate genotype

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¹Editor's note: This article and the two immediately following present three contrasting views of a topic of considerable importance to human genetics. In the first article Dr. Sanghvi criticizes a method of evaluating the genetic load that was first presented by Morton, Crow and Muller (1956) and later elaborated by Crow (1958), and a contrasting method is presented. In the second article Professor Crow clarifies and defends his previous position. The discussion is continued in the third article by Professor C. C. Li, who elaborates certain conclusions published (Li, 1963a, b) after Dr. Sanghvi's paper was submitted that are similar to those of Dr. Sanghvi.

It should be noted that the differences expressed are primarily philosophical rather than statistical, and are concerned with the proper choice of definitions for setting up mathematical models for examining biological systems. The usefulness of such statistical models lies in their ability to detect and discriminate specific components contributing to a complex biological mechanism. It is hoped that these discussions will serve to emphasize both areas of agreement and disagreement in theory, and will contribute to the development of a more unified theory.

²Present address: World Health Organization, Palais des Nations, Geneva, Switzerland. Dr. Sanghvi is on leave from the Human Variations Unit, Indian Cancer Research Center, Bombay, India.

(heterozygous individuals Aa) over the other two types (homozygotes AA and aa). Genetic loci which maintain deleterious traits by this mechanism have been termed "segregational loci" to distinguish them from the former ones which are termed "mutational." Interpretation of the maintenance of any fraction of the hereditary burden along this line would reduce the significance of radiation hazards to a corresponding extent. It has thus become an important question in population genetics as to whether we can distinguish the burden maintained by recurrent mutation from the portion that does not primarily depend on this process.

Any answer to this question based on observations in man is likely to come very slowly if current activity is any guide to future course. Morton, Crow and Muller (1956) attempted to assess the relative importance of these two mechanisms and concluded, by applying an original scheme, that most of the hereditary burden revealed by inbreeding is supported by recurrent mutation. The scheme, described in detail by Crow (1958), makes use of a property of differential response of mutational and segregational loci to inbreeding effects. Morton (1960) strengthened this conclusion by applying the same argument to the results of inbreeding in the parents of individuals afflicted with muscular dystrophy, deaf-mutism, and low-grade mental defect. On the other hand, Neel and Schull (1962) have presented recent data from Japan indicating that inbreeding effects are consistent with a larger component of segregational loci in that population.

These evaluations are based on acceptance of the theoretical scheme suggested by Crow. There are, however, conceptual and other difficulties involved in accepting this scheme. The purpose of this communication is to outline these difficulties and present an alternative approach for reconsideration of the problem.

THE CONCEPT OF GENETIC LOAD

There are several ways in which the term genetic load has been used in recent years. Muller (1950) used the term to express the proportion of population suffering genetic elimination or the amount of disability suffered by the average individual. Morton, Crow and Muller (1956) used the term "genetic damage," which implied the same general idea. Crow (1958) gave this idea a formal definition and some rules of operation. He defined the genetic load of a population as the proportion by which the population fitness (or whatever other trait is being considered) is decreased in comparison with an optimum genotype. He further stated that the genetic load has many possible components like the mutation load (genetic load due to mutational loci), the segregation load (genetic load due to segregational loci) and others. Crow (1960) has modified this definition as follows: the genetic load is defined as the proportion by which the average fitness in the population is decreased in comparison with what it would be if the factor under consideration (mutation) were absent (see also Fraser, 1962). Dobzhansky (1957) and Wallace and Dobzhansky (1959) suggested that deleterious mutant genes of all kinds constitute the genetic load of a population. As the idea has not yet reached the stage of any different

quantitative formulation from the one suggested above, it will not be discussed further in this communication.

MEANING OF GENETIC FITNESS

The genetic load as defined by Crow measures a decline in the average fitness of the population as compared to some "standard" population. The standard according to the first definition is a population of one optimum genotype and according to the modified version is a population under some specified condition, *e.g.*, a population in which no mutation occurs. For our present purpose, it is immaterial which definition we take. Both relate to a decline in average fitness of the population and it will, therefore, be appropriate for us to understand the term fitness, first.

The fitness of a population, considered over a large number of generations, determines its numerical strength. If the value of fitness is greater than unity, it means an expanding population. If it is less than unity, it is a declining population. If the estimates prepared by the United Nations (1958) about the size of the human population since the beginning of the Christian Era are accepted, they would tell us that we were increasing at the rate of about 1 per cent per generation at the beginning of the Era. This rate increased to about 10 per cent in the seventeenth century and we are currently increasing at a rate of about 50 per cent per generation. In terms of fitness, the value which was 1.01 at the beginning of the Era is currently running at 1.50 per generation. We assume here that the population at a particular time produces the next generation at an interval of 25 years. It is not necessary to enter into some of the complexities of the real situation resulting from overlapping generations and the age structure.

This rate of increase, described by Malthus as the law of geometric increase of population, bears a close analogy with the growth of capital invested at compound interest, $(1 + m)^t$, where m could be taken as the rate of increase and t is the number of generations. When m is small and t becomes large,

$$(1 + m)^t \rightarrow e^{mt},$$

an expression used by Fisher (1930) to develop a related concept of reproductive value. He termed m the Malthusian parameter of population increase. A negative value of m would mean a decline in population number.

The fitness of a population can alternatively be considered as an average fitness of individuals of this population. The fitness of an individual (or a genotype) is measured (Haldane, 1949; Penrose, 1949) by the number of its progeny, different generations being counted at the same stage of the life cycle (say, at birth). Fitness is thus concerned with the survival and reproduction of the individual. A tacit assumption in this extension of the idea of fitness from the population to individuals at a particular point of time is a stationary value of m over the span of generations covered by the birth of the oldest individual in the population to the time when all of them including the youngest had produced their last offspring.

If we are interested in changes of the composition of a population, we need only consider the relative fitness of different groups of individuals or genotypes. In the following argument we are not only concerned with relative fitness of

different genotypes of a population but also with relative fitness of populations under different systems of mating. This distinction should be carefully noted.

Fitness, as thus used, is strictly in the Darwinian sense of "success in leaving progeny" and is not intended to measure any other attribute of the individual or the population.

THE SCHEME SUGGESTED BY CROW

Crow's scheme is presented in table 1. Selection coefficients s , t_1 , and t_2 , as well as h , are all positive quantities and take values between zero and one. To illustrate the components of the genetic load, a numerical illustration is given in the lower portion of the table. It is chosen to have a frequency of $q = .005$ for the rare gene which corresponds to an incidence of 1 in 40,000 for a deleterious trait like phenylketonuria in a random-mating population. The fitness of the afflicted recessive homozygote is taken to be zero, and this may mean inability to survive or inability to reproduce.

The models under the scheme are based on assumption of a stable gene equilibrium from one generation to the next. Equilibrium in the mutation model is maintained by the appropriate amount of recurrent mutation from A to a to compensate for loss of a alleles. The condition of equilibrium in the segregation model under random mating requires that

$$\frac{t_1}{t_2} = \frac{q}{p}$$

giving $t_1 = \frac{.005}{.995} = .005025$ when $t_2 = 1$. The value of s under the mutation model is unity and the value of h is taken to be .005 to correspond closely with the value of t_1 . The chief result of Crow depends upon the ratio

$$\frac{\text{Genetic load under inbreeding (Inbred load)}}{\text{Genetic load under random mating (Random load)}}$$

which is $\frac{sq}{2hspq + sq^2} = \frac{1}{2hp + q}$ under the mutation model

and is $\frac{t_1p + t_2q}{t_1p^2 + t_2q^2} = 2$ under the segregation model.

For the numerical illustration in the table, this ratio comes out to be 67 for the mutation model and 2 (as expected) for the segregation model. The argument has been generalized to cover other human loci including those with multiple alleles. A large value of this ratio (say, 15), which is often referred to as the B:A ratio criterion, has been used as an evidence that segregational loci do not make any substantial contribution to our hereditary burden (Morton, Crow, and Muller, 1956) and a smaller value is taken to be consistent with a larger component of segregational loci (Neel and Schull, 1962).

Random Mating

Let us now examine the component parts of this genetic load. We will start with the population under random mating.

In the mutation model, the standard of comparison for calculation of genetic

TABLE 1. THE SCHEME SUGGESTED BY CROW (1958) WITH A NUMERICAL EXAMPLE

		Random Mating ($F = 0$)				Inbreeding ($F = 1$)			
		Mutation Model		Segregation Model		Mutation Model		Segregation Model	
Genotype		AA	Aa	AA	Aa	AA	aa	AA	aa
General case	Frequency	p^2	2pq	p^2	2pq	p	q	p	q
	Relative fitness	1	1 - hs	1 - t_1	1	1	1 - s	1 - t_1	1 - t_2
	Genetic load	0	2hspq	t_1p^2	0	0	sq	t_1p	t_2q
	Total load	2hspq + sq^2		$t_1p^2 + t_2q^2$		sq		$t_1p + t_2q$	
Specific case ($q = .005$)	Frequency	.990,025	.009,950	.990,025	.009,950	.995	.005	.995	.005
	Relative fitness	1.0	0.995	.994,975	1.0	1	0	.994,975	0
	Genetic load	0	.000,050	.004,975	0	0	.005	.005	.005
	Total load	.000,075		.005		.005		.010	

load is a hypothetical population consisting of individuals of *AA* genotype, an optimum under this model. The genetic load is contributed by the heterozygotes *Aa* and the deleterious homozygotes *aa*. The contribution of *Aa* to this load is twice that of *aa* for the value of $h = .005$. It will be 8 times and 20 times for values of $h = .02$ and $.05$, respectively.

In the segregation model, our standard of comparison changes. It is now a nonexistent population consisting of individuals of genotype *Aa*, which is optimum under this model and the genetic load is contributed by the genotypes *AA* and *aa*. The genetic load contributed by *aa* remains the same under both models. The genotype *AA*, which has become relatively slightly reduced in fitness under this model, contributes an enormous amount of genetic load—199 times the load contributed by *aa*. Thus, at the end, the genetic load under the segregation model comes out to be 67 times the load under the mutation model. It may be pointed out that such a difference in the genetic loads under the two models exists not because of any real demonstration of an inferiority of the segregational loci in natural populations, but arises as a result of the way in which the genetic load is defined. In other words, it is an algebraic artifact.

Inbreeding

When we examine the component parts of the genetic loads under inbreeding, the artifact becomes even more obvious. We observe that the load contributed by the genotype *aa* increases 200 times from a value of .000025 to .005 under both models. The load contributed by *Aa* under the mutation model disappears; and finally, the large amount of load contributed by *AA* in the segregation model changes quite insignificantly from a value of .004975 to .005. The net result is that the real inbreeding effect under the segregation model, which is not substantially different from that under the mutation model, is obscured by this enormous weight attached to the homozygote *AA*.

Another difficulty in the scheme arises from the fact that for the segregation model the selection coefficients t_1 and t_2 cannot remain stationary under inbreeding, but change according to the following formulae for gene equilibrium (Li, 1955):

$$\frac{t_1}{t_2} = \frac{q + Fp}{p + Fq}.$$

When $F = 0$, $\frac{t_1}{t_2} = \frac{q}{p}$ as we have taken; but

when $F = 1$, $\frac{t_1}{t_2} = 1$.

In other words, if the gene frequency is to remain stationary, the deleterious genotype *aa* and the genotype *AA* have to have the same fitness when $F = 1$. The ratio (inbred load:random load) thus will not be 2, but will be large under all conditions except when *aa* becomes almost as harmless as *AA* under inbreeding.

A CRITIQUE OF THE CONCEPT OF GENETIC LOAD

The authors of the concept of genetic load have not stated explicitly their main purpose in developing it. If the paper of Muller (1950) is any guide to its

origin, one of the purposes was probably to bring to focus the role of mutation in human morbidity and mortality. The concept will retain its validity if applied to loci with mutants having deleterious effects under all combinations of genotype and variations of environment. The concept will, however, remain of theoretical interest, so long as such mutants cannot be separated out in practice.

From a social point of view, a useful concept would have been the one which takes into account the contribution of various genotypes to the maintenance and progress in relation to their cost to the human society, along the lines ingeniously developed by Wright (1960). From a humanistic point of view, a useful concept should separate the components of fitness like survival and reproduction and assign appropriate weights (depending on cultural values) to these components and their subcomponents according to the hardship and human suffering they entail. The concept of genetic load as currently formulated does not take such views in account although it may have originated with such intentions.

On the other hand, if the purpose in developing this concept was a more general one, as it appears from the paper of Crow (1958), such as to understand the respective roles of mutational and segregational loci in natural populations, the concept has failed.

The standard of comparison chosen to measure a decline in fitness as originally defined by Crow (1958) cannot be accepted. A genotype with optimum fitness in the Darwinian sense is the one whose contribution to future generations is optimum strictly in terms of the number of descendants it leaves. In a humanistic or a social sense, such a genotype may not necessarily be optimum for any other objective consideration of human progress. Crow's revised definition (1960), where an attempt was perhaps made to rectify this defect, runs into trouble when applied to the segregation model. Under this model, it is difficult to find out which "factor is under consideration." Is it the segregation of alleles? Not really, since they are also segregating under the mutation model. Is it the advantage of the heterozygote? In that case, the standard population will consist of normal homozygotes not acceptable under the first definition.

Furthermore, the concept is developed in such a fashion that it puts the segregational loci at a disadvantage to start with. This is accomplished by not accepting any advantage of the heterozygote under the same working model. The artificial nature of this dichotomy of the mutation and segregation models may perhaps become more evident by a consideration of a general scheme outlined below. This scheme not only covers the mutation and segregation models as its particular cases but reveals a class of loci not covered by either of these models.

GENERAL SCHEME

The fitness of genotypes Aa and aa is considered as relative to the fitness of AA , which is taken to be unity. The coefficient of inbreeding F is incorporated in the genotypic frequencies. The scheme stands as follows:

Genotype	<u>AA</u>	<u>Aa</u>	<u>aa</u>
Frequency	$p^2 + Fpq$	$2pq(1 - F)$	$q^2 + Fpq$
Relative fitness	1	$1 + Kt$	$1 - t$

$$\begin{cases} 0 < t \leq 1 \\ Kt \geq -1 \end{cases}$$

The scheme gives the mutation model of Crow by substituting the relations $K = -h$ and $t = s$. Genetic load and mutation rate at equilibrium are given by the following formulae:

$$\text{Genetic load} = tq(q + Fp) - 2Ktpq(1 - F)$$

$$\text{Mutation rate} = tq(q - K + 2Kq)(1 - F) + Ftq.$$

The scheme covers the segregation model of Crow by noting the following set of relations obtained by equating $1 - t_1 : 1 : 1 - t_2 = 1 : 1 + Kt : 1 - t$.

$$K = \frac{t_1}{t_2 - t_1}; t = \frac{t_2 - t_1}{1 - t_1} \text{ or } t_1 = \frac{Kt}{1 + Kt}; t_2 = \frac{t(1 + K)}{1 + Kt}$$

In the absence of mutation, the condition of equilibrium requires that

$$\frac{t_1}{t_2} = \frac{q + Fp}{p + Fq} = \frac{K}{1 + K}.$$

The main difference between the result of Crow and the one given here arises in the measure of genetic load for the segregation model. Under the present scheme for random mating ($F = 0$), the change in fitness is

$$tq^2 - 2Ktpq = -\frac{tq^2}{1 - 2q} \text{ when } K = \frac{q}{1 - 2q} \text{ for equilibrium.}$$

This result, which amounts to a "negative genetic load," shows that the average fitness of the population in this case is slightly greater than unity and is based on the premise that a small advantage in fitness of Aa over AA can compensate for the loss of a alleles. For inbreeding, the average fitness is lowered by tq , a value also obtained for mutational loci under this scheme.

The apparent contradiction in the results of the two schemes can be resolved if we consider the variation in fitness of the population under inbreeding in relation to its initial fitness under random mating. The point is illustrated in table 2 by the numerical example considered before.

It will be seen from the table that the changes in average fitness of the population as a result of inbreeding are identical under the two schemes as would be expected. In other words, the appropriate test criterion should be $1-B/1-A$ and not B/A . One of the unexpected results that comes out rather clearly is that although the inbreeding effect is generally comparable for the two types of loci, it is slightly worse for the segregational ones. It may be indicated here that it is not essential to take the relative fitness of AA as unity under this general scheme. This value could have been assigned to any one of the other two genotypes.

An interesting outcome of this general scheme is to disclose more clearly the loci in which heterozygotes have a nominal advantage, but still require a substantial amount of mutation to maintain gene equilibrium at a given level. A numerical example is given in table 3 to illustrate this point. The table indicates that the classification of loci on the basis of relative fitness of the heterozygote is artificial to some extent, as it does not reveal any abrupt differences in behavior with respect to mutation rates.

There are three points which require some comment in relation to this general scheme. The first is the condition of equilibrium. Although this condition has shown itself to be a very convenient tool for theoretical reasoning, it is a question

TABLE 2. DIFFERENTIAL INBREEDING EFFECTS OF THE MUTATIONAL AND SEGREGATIONAL LOCI ON THE AVERAGE POPULATION FITNESS

	Crow's Scheme					
	Mutation model			Segregation model		
	F = 0	F = 1	F = 0	F = 1	F = 0	F = 1
Genetic Load	.000,075	.005	.005	.01	.000,075	.005
Average population fitness	.999,925	.995	.995	.99	.999,925	.995
Population fitness for F = 1 relative to that for F = 0	0.995,075			0.994,975		
				0.995,075		

*Explanation in the text.

TABLE 3. MUTATION RATES ($\times 10^{-5}$) FOR DIFFERENT VALUES OF RELATIVE FITNESS OF THE HETEROZYGOTE AS GIVEN BY THE GENERAL FORMULAE $tq(q - k + 2Kq)(1 - F) + Ftq(q = .005; t = 1)$

Fitness of Heterozygote Relative to Normal Homozygote									
0.98	0.99	0.995	0.997	0.999	1.0	1.001	1.003	1.00505	1.01
									1.01525
F = 0	12.40	7.45	4.98	3.99	3.00	2.50	2.01	1.02	0
F = .01	17.28	12.38	9.93	8.95	7.97	7.48	6.99	6.01	5.00
Mutation Model					Possibilities not Covered by These Models				
					Segregation Model				
					2.58				
					0				

of some practical importance as to how long such conditions prevail in natural populations. The number of generations required to approach equilibrium in certain cases is so large that major changes in environment may vitiate any long-term interpretation.

The second limitation to a theoretical formulation arises out of our very static concept of environment. We recognize, in principle, that development of an individual is an interaction between his genotype and his environment. Detailed genetic observations have led us to a fair amount of understanding of the possible array of genotypes in a population. On the other hand, our knowledge about the main components of our environment, their variation in time and place and their relationship with organisms is very limited. We still continue to think that any change in environment worth considering by geneticists could only occur over a span of hundreds of generations.

The third point relates to the heterozygote which is slightly different from the normal homozygote. We are somewhat in a position to measure the fitness of a frankly deleterious homozygote or heterozygote. In the case of a heterozygote slightly different from the normal homozygote there is no easy way; and it is here that environment and genetic background are both likely to have a proportionately larger effect in its expression. It is even possible that the spread of the values of relative fitness of the heterozygote is of greater consequence to the ultimate fate of the mutant allele than its average value. In this connection, it may be pointed out that the variate K used in the present scheme permits a better understanding for small values of q and F which are critical for a transition of some of the mutational alleles to become segregational ones and a gradual acceptance of rare useful ones amongst them as respectable members of our gene pool. For values of q beyond $\frac{1}{3}$, however, the ratio $t_1:t_2$ is more convenient to use.

INTERPRETATION OF INBREEDING EFFECTS

One of the difficulties in the scheme suggested by Crow has arisen out of the fact that some of the primary changes occurring under inbreeding have not been kept under view. Inbreeding reduces the frequency of heterozygotes and distributes equally to the two homozygotes. The homozygote which is rare has a proportionately bigger gain. No amount of formulation can alter this basic fact. If the frequency of two alleles at a locus are equal, inbreeding has equal effects on both homozygotes. This balance progressively changes as the frequency of one of the alleles gets smaller and smaller. The inbreeding effect is most profound on homozygotes determined by very rare genes.

If we are interested in the utilization of inbreeding effects for any rational genetic formulation, we should deal with genes which are very rare and exert their deleterious effects primarily through homozygotes. Whether the relative fitness of the heterozygote is slightly better than normal homozygotes or slightly worse cannot, in principle, be measured by inbreeding effects unless these effects are different in nature and distinguishable in practice.

The Hardy-Weinberg Law requires random mating and absence of mutation and selection in a closed population to maintain equilibrium. If we introduce

inbreeding in this population, in which we also add mutation and selection, we come out with unforeseen results. The numerical illustration in table 3 demonstrates these variable effects. For the mutational loci, a fixed value of K leads to an increase in mutation rates for maintenance of the equilibrium. For the segregational loci, inbreeding requires greater values of heterozygote advantage for maintenance of the equilibrium.

SUMMARY

Crow suggested a provocative scheme to evaluate the relative importance of mutational and segregational loci in maintaining our hereditary burden. The genotype used as standard of comparison in his mutation model is not the same as the one used in his segregation model. A logical approach will require a fixed standard of comparison for the two models. A scheme is outlined here in which the same genotype is used for evaluating the inbreeding effects on the two types of loci. It turns out that the appropriate test criterion for this purpose should be $1-B/1-A$ and not B/A as suggested by Crow. This and some other points related to the concept of "genetic load" are discussed.

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2. The Concept of Genetic Load: A Reply

JAMES F. CROW

*Department of Medical Genetics, School of Medicine,
University of Wisconsin, Madison, Wisconsin*

I AM GLAD TO ACCEPT the invitation of the Editor to reply to Dr. Sanghvi's article which appears in this issue of the *American Journal of Human Genetics*. I should like also to use this opportunity to answer an article by C. C. Li (1963a) in which some of the same points arise. Dr. Li's arguments are repeated in another paper (1963b). Since all three articles involve interpretation of formulae and the choice of definitions, I would have preferred to deal with these in private correspondence with the two authors. On the other hand, if some previously obscure points are clarified, this public exchange is perhaps useful.

The basic idea of the genetic load was first discussed by Haldane (1937) in a paper entitled "The effect of variation on fitness." In this paper Haldane showed that the decrease of fitness in a species as a consequence of recurrent mutation is approximately equal to the total mutation rate multiplied by a factor that ranges from 1 to 2 depending on dominance, inbreeding, and possible sex-linkage. On the basis of prevailing data on mutation rates, Haldane estimated that the average fitness of the population is decreased by about 5 per cent from this cause. The principle is remarkable in that the decrease in fitness at equilibrium does not depend on the degree of harmfulness of the mutant, provided that its average selective disadvantage in the various genotypes that comprise the population is large relative to the mutation rate and that it is deleterious or at best neutral in the heterozygous state. Essentially the same principle was discovered independently a few years later by Muller and was discussed fully in his 1950 paper, "Our load of mutations." Haldane also pointed out in his 1937 paper that for a locus in which the heterozygote is superior in fitness to either homozygote the average population fitness will be less than that of the heterozygote (although greater than either homozygote). He noted further that the decrease in population fitness, relative to the best genotype, is usually considerably larger for a locus whose variability is maintained by heterozygote superiority than for one maintained by recurrent mutation.

The decreases in fitness from these two causes have been designated as the *mutation load* and the *segregation load* (Morton, Crow and Muller, 1956; Crow, 1958). One can also consider the load that is ordinarily hidden by heterozygosis, but which would be revealed if the population were somehow made homozygous. This was called the inbred load and can be estimated by extrapolation from data on the progeny of parents of a specified degree of relationship, which are homozygous for a known fraction of otherwise heterozygous loci.

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¹See Editor's Note, page 298.

The expected ratio of the inbred load to the randomly mating load can be expressed in terms of meaningful genetic parameters. With a locus where two or more alleles are maintained by superior fitness of heterozygous combinations I have shown (1958, 1961) that the ratio is equal to or less than the number of alleles maintained in the population (or 2, if there are only two alleles). For a locus where the mutant alleles are maintained by mutation the ratio is approximately equal to the reciprocal of twice the average dominance, provided the mutants have sufficient dominance that most mutant gene eliminations occur through heterozygous effects. Data on dominance of *Drosophila* lethals suggest a ratio of about 20. For a completely recessive mutant the ratio is usually still greater, being $\sqrt{s/u}$, where s is the selective disadvantage of the mutant homozygote and u is the mutation rate. Thus, a high ratio is an argument that the majority of loci are mutational, or if segregational that a large number of mutually heterotic alleles are maintained in the population. These alleles must be sufficiently more advantageous in heterozygous combinations than in homozygotes that a large number are maintained in a stable polymorphism, despite the loss of alleles by random drift which increases roughly as the square of the number of alleles (Kimura, 1955, 1956), a circumstance that Morton, Crow and Muller (1956) regarded as unlikely for more than a minority of loci.

In the special case of the segregation load where there are only two alleles (the case treated by Li and Sanghvi), the ratio of the inbred to the random mating load is two. Li says that this is an artifact of my particular system of notation. However, the ratio as I have defined it is the same in any notation. The genetic load was defined in the paper Li refers to (Crow, 1958) as "the proportion by which the population fitness (or whatever other trait is being considered) is decreased in comparison with an optimum genotype." If w_m is the fitness of the genotype with the highest average fitness, the genetic load of a randomly mating and an inbred population are defined in Li's terminology as:

$$L_o = \frac{w_m - \bar{w}_o}{w_m}, L_1 = \frac{w_m - \bar{w}_1}{w_m}, \frac{L_1}{L_o} = \frac{w_m - \bar{w}_1}{w_m - \bar{w}_o}$$

where the subscript 0 means random mating ($F = 0$) and 1 means completely homozygous ($F = 1$).

Using Li's (1963a) "System I" notation, which he says gives a different result, $w_m = 1 + Hs$, $\bar{w}_o = 1 + Hsq$, $\bar{w}_1 = 1 - sq$, and the equilibrium value of $q = H/(1 + 2H)$. Substituting these into the above equations leads to $L_1/L_o = 2$, as expected. The quantity L_1/L_o , as I have defined it, is just as "invariant" with respect to changes in notation as the ratio, \bar{w}_1/\bar{w}_o , that Li and Sanghvi advocate.

Since, as emphasized in my paper, the load involves only the relative fitnesses of the three genotypes and not their absolute values, I arbitrarily assigned the value 1 to w_m . This was done solely for algebraic convenience, and I hope no reader was led to think that this value has some absolute meaning.

Sanghvi makes the point on page 303 that if two homozygotes are of unequal fitness, most of the segregation load is attributable to the less harmful homozygote. I agree, and have already emphasized the same point (Crow, 1961; Hirai-zumi and Crow, 1960). Sanghvi goes on to say that for this and other reasons the segregation load as I have defined it is an "algebraic artifact" and argues that

the reference point should not be the best genotype, but rather the best *homozygous* genotype. This is, of course, one possible definition; but it should be no surprise that a different definition leads to different results.

I counted myself fortunate in having found a simple relationship, L_1/L_0 , which has a strikingly different value for two kinds of gene loci (a locus where harmful alleles are maintained by mutation as contrasted to a small number of alleles maintained by heterozygote superiority), and which therefore offers the possibility of distinguishing between them experimentally. Li and Sanghvi say that the ratio \bar{w}_1/\bar{w}_0 is more appropriate. It may be for some purposes, but not for this, for its value usually differs only slightly for the two gene models and doesn't have (for me, at least) any such transparent interpretation in terms of allele numbers and average dominance, as does L_1/L_0 . Therefore it provides no basis for distinguishing between these two contrasting models of inbreeding effect.

Li says that I wrongly conclude that "population fitness decreases on inbreeding to a much greater extent for a mutational equilibrium population than for a heterotic one." I reached no such conclusion. My conclusion was rather the one that I have just discussed, which is that the *ratio* of the inbred to the random *load* is greater for a mutational locus than for a segregational one. (My 1958 paper has a carelessly worded statement at the bottom of page 10 that when taken out of context may be misleading. I said: "Unless the segregation load is based on loci with a large number of alleles, maintained in balanced polymorphism, it is not changed much by inbreeding. In general, I would conclude that if the fitness is greatly decreased by inbreeding it is to that extent largely attributable to the mutation load rather than the segregational." I should perhaps have said the fitness decrease *relative to the random mating load*, but I thought this would be clear from the overall context, and in particular from the preceding sentence. If this sentence is the source of Dr. Li's misunderstanding, I apologize to him and to any other reader who was confused by it.)

Sanghvi implies that I was in error in not taking into consideration the change of gene frequencies by selection during inbreeding. On the contrary, I stated explicitly in the 1958 paper and in subsequent papers that the L_1/L_0 ratio can be interpreted in terms of allele numbers and dominance only if the inbreeding occurs without a change in gene frequencies in a population that has reached equilibrium under the previous mating system. For this reason, my colleagues and I (Morton, Crow and Muller, 1956; Morton, 1960; Greenberg and Crow, 1960) have been careful to apply the theory only to populations where all the inbreeding occurred in a single generation, thus permitting no gene frequency changes. The methods are not applicable (without considerable modification) for populations where there is a history of inbreeding over several generations, as in most domestic livestock.

Both Li and Sanghvi believe that the standard of reference for the segregation load should not be the best genotype, as I have chosen, but rather the best homozygous genotype. However, the relationships that I have just pointed out depend on the proper definition. Other definitions are of course possible, but I would urge that the words "genetic load" be restricted to my definition and that other words be substituted when the definition is changed. To change definitions

at this stage leads only to confusion. I should also emphasize that the relationships that I have discussed are applicable only to data that are measured in a way appropriate to the definition. For example, since the measurement is from an optimum genotype the applicability is to data where there is a clear optimum—for example, premature death rate where the optimum is obviously zero (Morton, Crow and Muller, 1956).

To me it has always seemed natural on other grounds as well to measure the segregation load as a deviation from the best genotype, not from the best *homozygous* genotype. With a large number of alleles, the choosing of an inferior genotype simply because it is homozygous seems more arbitrary. Also, I have difficulty in defining the load for a balanced lethal using a homozygote as the reference point. In a later paper (1961) I suggested another definition: the genetic load is the proportion by which the average fitness (or other trait) is decreased in comparison with what it would be if the factor under consideration were absent. This was not (as Sanghvi suggests) an attempt to rectify any defect in the earlier definition, but rather to view the question from another perspective. With this definition the choice of the best genotype (in this case a heterozygote) as the reference point for the segregation load is clear; in a population without segregation (*i.e.*, reproducing asexually) the best genotype, heterozygote or homozygote, would replace the others.

However, the value of a definition is mainly determined by its ability to aid in the discovery of new relationships. Besides the L_1/L_0 test already referred to, our definition leads to a method of estimating the segregation load for any number of alleles from information on the fitness and frequency of any one of them (Morton, 1960; Crow, 1961). It has also been useful in more difficult problems such as arise with maternal-foetal incompatibility (Crow and Morton, 1960), selection for an intermediate metrical phenotype, and the effects of finite populations and varying environmental conditions (Kimura and Crow, unpublished).

On the other hand, I do not want to make any exaggerated claims for the method. It may turn out that the assumptions are too restrictive to be applicable to many real situations. The L_1/L_0 ratio suffers from the fact that while a high value suggests mutational loci as the major cause of inbreeding decline a low value offers no evidence for the contrary hypothesis, since it may simply be due to errors of measurement, to a mixture of dominant and recessive mutants, to inflation of the denominator by a large non-genetic component, or to other irrelevant factors. The theory applies only to traits that are highly correlated with fitness (strictly, only to fitness itself). The L_1/L_0 criterion does not apply when selection of different genotypes is on different traits or on different parts of the life cycle. For example, in a two allele segregational locus if one homozygote has a high death rate while the other has a low fertility, a study of death rates alone would be misleading. For this reason, Morton (1960) argued for mutational rather than segregational maintenance of alleles for mental deficiency primarily because of the large value of L_0 rather than because of a high L_1/L_0 ratio. Another difficulty is that a population in a heterogeneous environment and with division of labor may not have an optimum genotype with respect to some loci. In other situations, for example in Wright's theory of evolution in

a subdivided population, the average fitness and genetic load may not be the most important considerations from a long time view. Finally, there are many difficulties and deficiencies at the data level, and as emphasized before, the data used must be consistent with the assumptions of the model. Despite such difficulties, I believe the methods when used correctly are helpful for a number of problems in population genetics.

My own views of population structure are misinterpreted by Sanghvi on page 301. Morton, Muller and I (1956) did not regard our analysis as "evidence that segregational loci do not make any substantial contribution to our hereditary burden." If Sanghvi means, as I think he does, the *expressed* hereditary burden, our study offered very little evidence on this point; what we did suggest from the high L_1/L_0 ratio was that such loci make a relatively small contribution to the *hidden* load revealed by inbreeding. I have argued long ago (1948, 1952) for the importance of overdominant loci and other selectively balanced systems in population fitness. But, because of their tendency to accumulate homozygous-detrimental alleles in the population at high frequencies, such loci exert an influence on population fitness out of all proportion to their number, and therefore a large effect could be caused by a relatively small number of loci.

The effect of mutation on the normal incidence of disability and on fitness in general is very difficult to assess from ordinary data, because of the confounding effects of such things as loci maintained by selective balance and non-genetic effects. Except for specific traits that are amenable to segregation analysis it is almost impossible to assess the mutational component. On the other hand, the inbred load is influenced relatively more by the kind of mutant whose frequency depends on the mutation rate than is the random load, so the study of consanguineous matings is a way of making an assessment of mutational effects that is less contaminated from segregational loci than if it were based on the study of progeny of unrelated parents.

There are a number of other points where I believe that Li and Sanghvi are in error, or have misunderstood our papers, but these are of less general interest and are best discussed in private correspondence.

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3. The Way the Load Ratio Works

C. C. LI

*Department of Biostatistics, University of Pittsburgh,
Pittsburgh, Pennsylvania*

THE CONCEPT OF GENETIC load and the method of load ratio have been an interesting and popular subject in population and human genetics for the last few years as judged by the large amount of literature citing the concept and the method. The open discussions of Sanghvi and Crow, presented in the foregoing pages, are most timely, and they have undoubtedly contributed a great deal toward clarification of the method of load ratio. In every frontier of science there are disagreements and controversial subjects, and genetics, being in a healthy condition, is no exception. I believe that it is through these discussions that we make progress in formulating our concepts and in devising our methodology. At this stage, it is not at all a matter of right or wrong. The history of science shows that scientists often start with "wrong" ideas and as knowledge accumulates they gradually modify original concepts and reach better ones. From the historical viewpoint, an original "wrong" paper may contribute just as much as a final "correct" one. In the present case, it happens that I have also discussed the problem of genetic load elsewhere (Li, 1963 a, b). The only reason that I write this rejoinder is my belief that open discussions will benefit, not harm, everyone concerned, in the long run if not immediately. In the following I shall confine myself to the main features and implications of the load ratio. As brief as it is, I shall try to make this communication more or less self-contained so that it may be read independently without constantly referring to other papers.

THE BASIC PROBLEM

Let w_1 , w_2 , w_3 be the relative fitness of genotypes AA , Aa , aa , respectively, and let us consider the case in which w_3 is much smaller than w_1 and w_2 ; that is, the genotype aa leads to the development of a serious disease or condition that impairs reproductive ability (not necessarily involving deaths). We then say that selection is against genotype aa . In an equilibrium population, there must be some mechanism of compensation for the loss of gene a through selection. Indeed, there could be many different kinds of compensation in nature, about which, unfortunately, we know too little. However, if $w_1 > w_2 > w_3$, the simplest compensation mechanism is recurrent mutation from allele A to allele a . We shall call this a "mutational" equilibrium for brevity. On the other hand, if $w_1 < w_2 > w_3$, the higher reproductive ability of the heterozygote (Aa) will maintain the gene a in the population without the help of new mutations in

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¹See Editor's Note, page 298.

every generation. We may call this a "heterotic" equilibrium for brevity. Since we deal with the case in which w_3 is much smaller than w_1 and w_2 , the type of equilibrium is essentially determined by $w_1 > w_2$ or $w_1 < w_2$. The two types of equilibria mentioned above are by no means the only possible ones in nature but we shall confine our discussion to these two types.

Now, consider a random mating population in which the frequency of gene A is $p = .98$ and that of gene a is $q = .02$. The population consists of, phenotypically,

$$.9604 + .0392 = .9996 (AA + Aa) \text{ and } .0004 (aa).$$

Genotypes AA and Aa are all apparently normal, and even if they are different to some slight degree in their fitness values, they are certainly indistinguishable without detailed direct studies. The basic problem is: what type of equilibrium is this? Mutational or heterotic? There is no way to tell by looking at the genetic composition of the population. Since genotypes AA and Aa are hardly distinguishable, the relative magnitudes of w_1 and w_2 are totally unknown. If we render the population completely homozygous (actually by partial inbreeding and extrapolation) without changing the gene frequencies, the population would consist of

$$p = .9800 (AA) \text{ and } q = .0200 (aa).$$

The most conspicuous result of complete inbreeding is that the proportion of aa has increased from .0004 to .0200; or $q/q^2 = 1/q = 1/.02 = 50$ times. This will be so whether the equilibrium is mutational or heterotic as the genes remember no history (the Markov property). All that sudden and complete inbreeding does is to transform the genotypic distribution ($p^2, 2pq, q^2$) to ($p, 0, q$), whatever the type of the equilibrium. This is one of the arguments advanced by Li (details in 1963b) who concluded that inbreeding cannot help us to distinguish the two types of equilibria; in other words, inbreeding is no tool to diagnose why deleterious genes are in the population or how they are maintained there. Crow's (1958, 1963) contention is that inbreeding results can distinguish mutational equilibrium from an heterotic one, and this is accomplished by using a quantity known as "genetic load." Let us now examine how it works.

THE LOAD RATIO METHOD

Adopting the notation of Crow (1963), we let $w_m = \max (w_1, w_2, w_3)$ be the largest of the three fitness values. The genetic load at the random mating state is $L_o = (w_m - \bar{w}_o)/w_m$ and that at the completely inbred state is $L_1 = (w_m - \bar{w}_1)/w_m$, so that the ratio of these two loads is

$$L_1/L_o = (w_m - \bar{w}_1)/(w_m - \bar{w}_o).$$

Crow (1958, 1963) showed that the value of this ratio is always equal to 2 for a population in heterotic equilibrium and the ratio is large for a population in mutational equilibrium. Thus, through inbreeding and calculating the value of L -ratio, one would be able to tell whether the equilibrium is maintained by

heterosis or by recurrent mutation. Table 1 gives a numerical illustration of the method using the population with $p = .98$ and $q = .02$ mentioned previously. There are an infinite number of possible heterotic equilibria that can maintain the frequency of the deleterious gene at the $q = .02$ level, but for the particular

TABLE 1. TWO MODELS OF EQUILIBRIUM (HETEROTIC, H; AND MUTATIONAL, M) WITH GENE FREQUENCIES $p = .98$ AND $q = .02$

Genotype	Frequency (random mating)	Frequency (completely inbred)	Population H fitness w	Population M fitness w
AA	.9604	.9800	980	1000
Aa	.0392	0	1000	980
aa	.0004	.0200	20	20
random mating	{	mean \bar{w}_0	980.400	998.824
		load L_0	19.600	1.176
completely inbred	{	mean \bar{w}_1	960.800	980.400
		load L_1	39.200	19.600
ratio of means		\bar{w}_1/\bar{w}_0	0.9800	0.9816
load ratio		L_1/L_0	2.00	16.7

numerical example chosen $w_1 : w_2 : w_3 = 98 : 100 : 2$. Likewise, there are an infinite number of possible mutational equilibria that can also maintain the level of the deleterious gene at $q = .02$, and I have chosen the situation $w_1 : w_2 : w_3 = 100 : 98 : 2$ for an example. It is important to note that $w_m = w_2$ in the former case, and $w_m = w_1$ in the latter. In calculating the genetic load (lower portion of Table 1), I have merely used the deviation $w_m - \bar{w}$, omitting the denominator w_m for simplicity, as this does not affect the value of the ratio L_1/L_0 .

From the numerical calculations it is clear that the mean fitness \bar{w}_0 of the random mating population is always close to w_1 whether the equilibrium is due to heterosis or due to mutation, simply because the great majority of the individuals in the population are of genotype AA. After eliminating the heterozygotes from the population, the new mean fitness \bar{w}_1 is about 2 per cent lower than \bar{w}_0 in both populations. Hence Sanghvi and Li do not think that the two types of populations react to inbreeding very differently. However, if one looks at the load ratio, he will find that it is exactly 2 for population H and 16.7 for population M. Based on this difference, Crow thinks that he can distinguish the two types of equilibria through inbreeding. The artificial nature of this comparison has been discussed by Sanghvi and will not be repeated here. Instead, I will raise an operational question, *viz.*, how could we ever calculate the value of a genetic load, not mentioning the ratio of two loads, without first knowing whether $w_m = w_1$ or $w_m = w_2$?

We should remember that the original problem was to determine the type of equilibrium or, equivalently, to detect whether $w_1 > w_2$ or $w_2 > w_1$ in the population .9996 (AA + Aa) and .0004 (aa) through inbreeding. Now, as exemplified in Table 1, in order to calculate a quantity that is different for the

two models, we must know *a priori* that $w_m = w_2$ in one case and $w_m = w_1$ in another. How could we know this when facing an unknown population like .9996 ($AA + Aa$) and .0004 (aa) ? There are 3.92 per cent heterozygotes in the population. How could we first isolate them? If we can separate AA from Aa , their relative fitness could then be studied directly and why we should resort to inbreeding at all? If we know $w_1 > w_2$ or $w_2 > w_1$, then there is no problem to begin with. In one word, *the method of load ratio requires the prior knowledge of the answer itself*. It cannot be applied as a tool of diagnosis to an unknown population. However, Crow (1958) reported that for a certain body of data on consanguineous matings, the load ratio is found to be 17, implying that it is a mutational equilibrium for the locus under consideration. The raw data and the step-by-step arithmetic procedure of arriving at this ratio have not been given and we have no way to tell precisely how values of w_m , \bar{w}_0 and \bar{w}_1 have been obtained.

The discussions of Li and of Sanghvi were written before Crow supplied an explicit expression for the load ratio. The essential "misunderstanding" on the part of Sanghvi and Li is that they employed w_1 of AA in place of w_m of an unknown genotype to measure the genetic load. It appears that this measurement is more realistic, at least in describing the population situation (see last section).

THE MORE SIMILAR, THE MORE DIFFERENT

In addition to the operational difficulty of the load ratio method, there is another property that is very curious, *viz.*, the more similar the two population models are, the greater the difference in their load ratios! To illustrate, let us consider the four pairs of populations in table 2. In the first pair (H_1 and M_1),

TABLE 2. THE RELATIVE FITNESS VALUE (w) AND GENE FREQUENCY (q) OF FOUR PAIRS OF POPULATIONS; H = HETEROTIC EQUILIBRIUM, M = MUTATIONAL EQUILIBRIUM

	$q = .100$ H_1 M_1		$q = .020$ H_2 M_2		$q = .004$ H_3 M_3		$q = .001$ H_4 M_4	
w_1	900	1000	980	1000	996	1000	999	1000
w_2	1000	900	1000	980	1000	996	1000	999
w_3	100	100	20	20	4	4	1	1
\bar{w}_0	910	973	980.4	998.8	996.016	999.952	999.001	999.997
\bar{w}_1	820	910	960.8	980.4	992.032	996.016	998.002	999.001
\bar{w}_1/\bar{w}_0	.901	.935	.980	.982	.99600	.99606	.9990	.9990
L_1/L_0^*	2	3.33	2	16.7	2	83.3	2	333

*ratio = $(w_2 - \bar{w}_1)/(w_2 - \bar{w}_0)$ for H populations.

ratio = $(w_1 - \bar{w}_1)/(w_1 - \bar{w}_0)$ for M populations.

the genotypes AA and Aa have a 10 per cent difference in their fitness values. A difference of such magnitude may be detected by direct studies as in the case of sickle cell trait versus homozygous normals. Yet, the load ratio for M_1 is so low (3.33) that it is not too different from the ratio value 2 for H_1 . The second pair is the same as that in table 1; the genotypes AA and Aa have only a 2 per

cent difference in their fitness values but the load ratio of M_2 is 16.7, much higher than the ratio 2 of H_2 . In the third pair, the fitness values of AA and Aa differ by less than 1/2 per cent and are probably indistinguishable by any actual studies, but the load ratios of H_3 and M_3 differ greatly. In the fourth pair, there is very little difference in fitness values between AA and Aa , but the load ratios for H_4 and M_4 are 2 and 333, respectively. If the method of load ratio is a valid tool for distinguishing the two types of equilibria, one must conclude that it is much easier to distinguish M_4 from H_4 than to distinguish M_3 from H_3 , which in turn is much easier than to distinguish M_2 from H_2 , and so on. In other words, the smaller the difference between w_1 and w_2 in the two types of populations, the greater the difference between them as measured by load ratios, and the greater the ease with which they can be distinguished through inbreeding. I thought the opposite should be true by any valid criterion of classification. I see very little difference between M_4 and H_4 and I would venture to say that it would be extremely difficult to tell which is in heterotic and which in mutational equilibrium by any method that may be devised.

THE MORE BENEFICIAL, THE MORE HARMFUL

Finally, we may examine the descriptive consequence of defining genetic load in terms of the maximum fitness value (w_m). Consider, as an example, a population of 1000 AA individuals; one of them becomes Aa through a new mutation. Suppose that the mutation is a favorable one, conferring directly a 2 per cent advantage in fitness on the heterozygote over the original homozygotes in example I and a 25 per cent advantage in example II:

Genotype	Frequency f	Fitness (I) w	Fitness (II) w
AA	.999	100	100
Aa	.001	102	125
aa	0	—	—
mean fitness	\bar{w}	100.002	100.025
load	$(w_m - \bar{w})/w_m$.01959	.19980
gain	$\bar{w} - w_1$.002	.025

If we use the fitness of genotype AA as the standard of comparison, we would say that in case I, the average fitness of the population has *gained* an amount .002 while in case II, the gain is .025 on account of the beneficial mutation. I think this is a reasonable description of the situation. However, if we calculate the genetic load in terms of the highest fitness value, the population will never have any gain no matter how beneficial the mutation is. In fact, the more beneficial the mutation, the greater the genetic load, implying that the population is suffering from a greater amount of genetic elimination and is worse off from an "optimum" genotype. Then it seems that the shortest way to alleviate the situation is not to have more favorable mutants but to kill off the existing one, so that there will be no selection and therefore no genetic load. This and other considerations has led both Sanghvi and Li to doubt if the expression $(w_m - \bar{w})/w_m$ has biological significance or usefulness as a descriptive index.

When considerations are extended beyond one locus, the concept of an "optimum genotype" will be even more unattainable and the average population fitness will play an increasingly important role, as stressed by Dobzhansky and Spassky (1963). The method of Sanghvi and of Li, allowing for both gain and loss and thus achieving a more realistic description of the population changes, is conceptually the same as that allowing for "genetic load" as well as for "genetic elite," as suggested by Dobzhansky and Spassky.

It may be argued (Crow, personal communication) that genetic load is designed to describe an equilibrium population, not to describe the effect of mutants. "That you can contrive a biologically nonsensical result by applying the definition in a circumstance for which it was never intended is no valid criticism of the theory." But there is no essential difference between describing the effects of mutants in a population and describing an equilibrium population. For instance, in a random mating population with $p = .9995$ and $q = .0005$, the genetic composition would be approximately

AA, .999

Aa, .001

aa, negligible

and this is in practice the same as having one mutant in every thousand individuals, and the same conclusion applies.

SUMMARY

The discussions presented in this communication may be summarized thus: Inbreeding results cannot distinguish one type of equilibrium from another. The method of load ratio is operationally backwards, as it requires prior knowledge of the type of equilibrium before calculating the ratio. The load ratio, if calculated, has the very unusual property that the more alike the two types of populations, the greater the difference in their load ratio and hence the greater the ease with which they can be distinguished. I doubt this very much. The definition of genetic load is such that the more beneficial the new mutant, the more harmful it is to the population, and this is hardly an accurate description of the effects of beneficial mutations. In my opinion, we need more, not less, discussion on the entire concept of genetic load and its possible applications so that we (pro and con) may eventually reach a better solution.

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LETTER TO THE EDITOR

ATYPICAL Fy^b IN SOME FAMILIES WITH A MONGOL CHILD

DEAR SIR,

In the September 1962 issue of this Journal we reported atypical inheritance of the Duffy system in three of 22 families in which there was a child with mongolism, in each case a Fy(a+b—) parent having a Fy(a—b+) child. Following this, and using the same antisera, we blood-grouped 53 more families in which there was a mongol child and among these families found two with the same Duffy anomaly.

In the meantime we had received as a gift from Mr. William Pollock of Ortho Research Foundation another anti-Fy^b serum, this one active only by the indirect Coombs technique. When the blood of the new Fy(a+b—) parents and control cells were now tested with all our anti-Fy^b sera we obtained the following results:

Test Cells	Dilutions of Ortho's Anti-Fy ^b							Two saline-active anti-Fy ^b "Plu." "J.S."	
	Neat	1/8	1/32	1/64	1/128	1/256	1/512		
True Fy(a+b—)	—20'	—20'	—20'	—20'	—20'	—20'	—20'	—	—
Negro Fy(a—b—)	—20'	—20'	—20'						
Parent Fy (a+b—)	+6'	+6'	+8'	+9'	—20'	—20'	—20'	—	—
Stand'd Fy(a+b+)	C<1'	C<1'	+1'	+2'	+6'	+8'	—20'	+	+

C<1' indicates complete agglutination in less than one minute, a powerful reaction; + 6' one that takes 6 minutes or more to develop, a very weak one; —20' indicates no reaction in 20 minutes. In Ortho's laboratory no reaction could be demonstrated between their serum and a "parent" Fy(a+b—) blood sent by mail. The essential points of the test results are (1) the parent type Fy(a+b—) cells do react in our hands, though very weakly, with the indirect Coombs anti-Fy^b; (2) such cells may at times be mistaken for true Fy(a+b—) cells; (3) true Fy(a+b—) cells and negro Fy(a—b—) cells do not react with this anti-Fy^b or the other anti-Fy^b sera available to us.

New specimens from three of the Duffy-anomalous parents of our September report were similarly tested with the same results (I-2 of Family 14, I-2 and II-2 of Family 18; blood has not yet been obtained from I-1 of Family 21). When the cells of one of these parents were exposed to the Ortho serum and an eluate made the eluate reacted specifically as anti-Fy^b.

It is evident that in these families we are not dealing with Fy, the "silent gene" so common in Negroes. What the explanation is for the weakness of the reaction with anti-Fy^b we do not know, nor do we think it worth discussing theoretical possibilities at this point. We feel, however, that the observation should be brought to attention.

We are most grateful to Mr. Pollock and his associates at Ortho Research Foundation for their gifts of a serum of such value and for the tests they carried out, and to Mrs. Jane Swanson, Minneapolis War Memorial Blood Bank for the

saline-active anti-Fy^b serum "J.S.". This work was supported by N. I. H. Grant RG-0750-02 and by the National Foundation (U. S. A.)

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BRUCE CHOWN, MARION LEWIS AND HIROKO KAITA
Rh Laboratory, 735 Notre Dame Ave., Winnipeg 3, Canada.

* * *

Book Reviews

New Patterns in Genetics and Development. By C. H. WADDINGTON. New York: Columbia University Press, 1962. (271 pp., 72 figs., 24 plates, \$10.00.)

This book is based on six Jesup Lectures given at Columbia University in 1961. The author states that, "The aim of this book is to consider the origin of new patterns of structure in developing cells and tissues in the light of these new patterns of thought" [in molecular biology].

The six chapters are: (1) "The Production of New Substances," a review of newer findings on DNA, protein synthesis, and gene regulation; (2) "Kinetic Organization and Cellular Ultrastructure," a discussion of epigenesis (in the Waddingtonian sense) and the formation of cell organelles; (3) "Types of Morphogenetic Processes," an attempt to classify processes in terms of the types of information involved, with a review of some nonbiological processes which resemble biological processes; (4) "Morphogenesis in Single Cells" and (5) "Multicellular Morphogenesis," both chapters consisting largely of brief discussions of a number of systems in which observations have been made and which evoke interesting questions of mechanism; (6) "Biological Patterns," a discussion of complex forms, the function of whose components can be described only in terms of the whole.

Professor Waddington has been a major contributor to the experimental as well as theoretical literature on morphogenesis. His interests have been wide ranging, a fact which is reflected in the variety of topics discussed. Few persons, however familiar they may be with some aspects of the subject matter, will fail to find new material presented. Waddington has given particular emphasis to recent investigations, even though—with the exception of chapter 1—there has been little change in our concepts of morphogenesis.

One of Waddington's strengths is his ability to handle abstractions in biology. But there are times when he carries this beyond the point of usefulness. The terms "canalization" and "creode" have not come into general use because they are so nonspecific as to be virtually meaningless. He also indulges excessively in retrospective insight, quoting from his own previous publications a number of times. This is reflected in the bibliography, which includes 29 references to publications in which Waddington is senior author. The runner-up is Sirlin with eight publications, two of which are co-authored by Waddington.

This book will be read with interest by those who are concerned with morphogenesis. It is not—nor was it intended to be—an introductory review. Persons without a special need to read the book may be discouraged by the turgid prose. (*H. Eldon Sutton*)

Genetics for the Clinician. By C. A. CLARKE. Philadelphia: F. A. Davis Co. (294 pp., \$8.50)

The title of this book raised the hopes of the reviewer that a current important need has been met. These hopes, however, have not been completely fulfilled by Dr. Clarke's book.

Although he incorporates sections on all the important aspects of human genetics, he has not been able to solve the problem common to many books on the subject. Many of the clinical and biochemical data are sketchy and well below the level of knowledge of today's clinician, while some of the statistical and theoretical chapters are unnecessarily complex for an introductory text. I believe that not too great a degree of re-writing with a correction of these disparities can transform this book into a valuable and useful orientation for the practitioner.

During such a revision, great care should be exercised in the elimination of a fair number of factual and theoretical errors, some of which are the following. On page 23, the statement is made that meiotic nondisjunction in the sex chromosomes of a male will produce only two types of abnormal offspring; if, however, second division nondisjunction is added to the listed first division nondisjunction, it will be seen that four different types of abnormal offspring can result. The assumptions on page 27 and 28 that genes on the Y chromosome cause the sex difference in the rate of development and that no feminizing genes are carried on the X chromosome should be qualified by stating for the former that these may well be secondary effects, and making some mention of the Lyon hypothesis for the latter. Dropping the proposition in the calculations in table III on page 38 is incorrect for this particular study since ascertainment here was from two affected parents and not from the offspring. The definition of the x(Bombay) gene as an inhibitor of the B gene does not fit with the current evidence that this gene prevents the synthesis of a substrate on which the B gene can act. The statement on page 44 that "genes probably never have one single effect" should be clarified that this refers to secondary phenotypic effects. On page 66, it is difficult to conceive how a B individual who has no anti-H antibody would be favored in a *Pasturella pestis* epidemic if this organism possesses an H-like antigen. The author asserts in several places that the Rh system is determined by at least three genes. Some mention of the other theory might be called for. The use of linkage with P, as mentioned on page 77, to determine which hemoglobins are allelomorphs seems unnecessary with modern biochemical techniques for peptide chain analysis. Incidentally, the defect in hemoglobin S is on the β -chain and not on the α -chain as stated on page 168.

As stated earlier, a careful revision would eliminate these errors and allow for greater stress on medical and biochemical genetics. The literary style, printing and artistic layout are excellent and it is hoped that a new edition of *Genetics for the Clinician* will fulfill its potential of being a very useful text. (Kurt Hirschhorn)

Discussions in Cytogenetics. By CHARLES R. BURNHAM. Minneapolis: Burgess Publishing Company, 1962. (375 pp., \$8.00).

This book is intended for advanced study in plant cytogenetics and assumes prior training in genetics and cytology. Five chapters are devoted to structural changes in chromosomes and three to changes in chromosome number. These are followed by chapters on polyploidy, sex determination and apomixis. Addenda include a section listing over 1,300 literature citations, an appendix of 87 problems and study questions and another of selected references for each chapter. Although animal cytogenetics are not treated extensively, this volume should prove a useful reference since it provides a fairly detailed account of classical studies in both theoretical and applied plant cytogenetics. (H. O. Goodman)

Approaches to the Genetic Analysis of Mammalian Cells: Michigan Conference on Genetics. Edited by DONALD J. MERCHANT and JAMES V. NEEL. Ann Arbor: University of Michigan Press, 1962. (97 pp., \$4.00)

These essays are based on a series of seven lectures presented at the University of Michigan during the spring of 1962. Their purpose was to explore the extent to which techniques developed in microbial genetics and in tissue culture investigations can and have been applied to genetic study of mammalian cells in culture. These interesting and informative presentations are: applicability of approaches of microbial genetics to characterization of mammalian cells, by Francis J. Ryan; drug sensitivity as a genetic marker for

human cell lines, by Wacław Szbalski and Elizabeth Hunter Szbalski; chromosome morphology as a genetic marker, by Paul S. Moorhead; biochemical and physiological markers, by S. G. Bradley; virus susceptibility of mammalian cells, by Joseph L. Melnick; antigens as genetic markers, by Leonhard Korngold; mating of somatic cells *in vitro*, by Boris Ephrussi and Serge Sorieul. The essayists are eminent investigators in their respective fields, and have presented stimulating and thought-provoking summaries. (C. N. H.)

Genetic Notes (Fifth Edition). By JAMES F. CROW. Minneapolis: Burgess Publishing Co., 1963. (157 pp., \$3.75, spiral ring paper bound)

These are the lecture notes of the excellent course in general genetics offered at the University of Wisconsin. When the book is open, the printed notes are on the left page, with the right page blank for the student to use in adding additional notes. The treatment of general genetic principles is extensive and well written. Problems to be solved by the student appear at the end of each chapter, and there is an appendix on statistical methods. These notes could be profitably adapted for use in similar courses in other universities. (C. N. H.)

Haemoglobin-Colloquium. Edited by HERMANN LEHMANN AND KLAUS BETKE. Stuttgart: Georg Thieme Verlag (U. S. Agents, International Medical Book Corporation, New York 16): 1962. (113 pp., \$7.40, paper-bound).

This Symposium on Hemoglobin was held on August 31, 1961, at Vienna in connection with the Eighth European Congress of Haematology. The 41 papers presented are divided into three sections: Structure of the Hemoglobin Molecule; Hemoglobin M and Other Variants; Thalassemia and Persistence of Fetal Hemoglobin. Each section is opened by a summary paper outlining previous work, and is followed by shorter papers presenting research results. About half of the papers are in English and the other half divided between German and French. Practically all of the papers are of genetic interest, and the volume gives a good picture of the status of hemoglobin studies as of mid-1961. (C. N. H.)

Problems of Hereditary Chondrodysplasias. By ANDREAS HOBÆK. Oslo University Press, 1961 (U. S. Agents, 553 North Street, Boston 9, Mass.) (175 pp. Price, \$6.50. Paper-bound).

Hereditary chondrodysplasia is used in this monograph as a collective term for the group of hereditary diseases characterized by anomalous cartilage formation. An extensive review of the literature is presented. The clinical and radiologic findings are described for patients in the following diagnostic categories: gargoylism, six families; infantile hereditary chondrodysplasia, general type, 12 families; infantile hereditary chondrodysplasia with mainly vertebral lesions, eight families; infantile hereditary chondrodysplasia with mainly epiphyseal lesions, 11 families; unclassifiable chondrodysplasias, four families; chondrodysplasia calcificans congenita, one family. A pedigree drawing is presented for each family, and there is some attempt to classify these as dominant, recessive, or sex-linked, but there is no genetic analysis. (C. N. H.)

Advances in Rheumatic Fever, 1940-1961. By MAY G. WILSON. New York: Hoeber Medical Division of Harper & Row, Inc., 1962 (249 pp., \$10.00)

The extensive long-term studies of the natural history of rheumatic fever carried out at the Cornell Medical Center by the group headed by Dr. May G. Wilson are well known. Her previous monograph, *Rheumatic Fever*, summarized the results for the years 1916 to 1940, and the present monograph presents the findings of the next 20 years of study. These are arranged in four sections dealing with epidemiology, nature of the disease, diagnosis and course, and management. The data on age susceptibility, intrafamilial spread of rheumatic fever, recurrence rates and their relationship to environmental factors,

genetic analysis of rheumatic families, and blood groups and secretor status in rheumatic fever are all of considerable interest to geneticists.

The genetic data are in agreement with the hypothesis that susceptibility to rheumatic fever is controlled by the homozygous state of a recessive autosomal gene. This conclusion is supported by analysis of 109 families with 456 children ascertained through an affected child, and also by a series of 291 families with 646 children ascertained through a selected parent. It is pointed out that a more complex genetic mechanism cannot be excluded. The work of other investigators is summarized, extensive bibliographies follow each chapter, and the index is adequate. This monograph is interestingly written, and should become a standard reference work on the epidemiology, etiology, and natural history of rheumatic fever and rheumatic heart disease. (C. N. H.)

Blood Groups and Transfusions (Reprint of Third Edition). By ALEXANDER S. WIENER. New York: Hafner Publishing Co., 1962. (438 pp., \$14.50)

The original Third Edition of Dr. Wiener's classic work on blood groups appeared in 1943; this reprint includes a new seven page introduction by the author. He mentions the overwhelming mass of literature on blood groups that has appeared in the past 20 years, and points out that most current books emphasize current research, and that much useful older information seems to have been lost by attrition. This reprint is offered as a method of making available some of the "lost" information not available in more modern texts. This volume should have historic interest to those working in many areas. (C. N. H.)

Experiments in Genetics with Drosophila. By MONROE W. STRICKBERGER. New York: John Wiley and Sons, 1962. (144 pp., \$3.95, spiral-bound).

This laboratory manual contains clear and detailed directions for student experiments with *Drosophila* ranging from the usual single and multiple gene crosses through experiments in quantitative inheritance, detection of lethals produced by irradiation, and population cage experiments. The supplementary material should be quite useful to students, including sections on the *Drosophila* life cycle, laboratory techniques, numerous excellent drawings of various mutation effects, a section on statistical treatment of data, and appendices listing and describing mutations and giving a linkage map and also a key for identification of wild species that may be encountered in the United States. There is also a selected list of references for parallel reading. (C. N. H.)

The Biosynthesis of Proteins. By H. CHANTRENNE. Oxford and New York: Pergamon Press, 1961. (220 pp., \$6.50)

The recent rapid advances in protein chemistry have made it difficult to produce an introductory textbook covering the entire field. Dr. Chantrenne's attempt has been successful in producing a readable text that is coherent and reasonably comprehensive. In some areas the treatment may not be sufficiently detailed for some readers, but a bibliography of 33 pages provides adequate selected source material. A picture is provided of the status of information concerning biosynthesis of proteins as of 1960, and a firm foundation is provided for understanding current developments appearing in the literature. (C. N. H.)

The Genetic Code. By ISAAC ASIMOV. New York: The New American Library (Signet Science Book), 1962. (187 pp., \$.60, paper bound)

The author is Associate Professor of Biochemistry at Boston University School of Medicine, and his output of popularized "science fact" articles in various periodicals is impressive. In his usual lively style, he has reviewed the development of knowledge concerning DNA and the progress made toward solving the genetic coding of protein synthesis. The book is intended for the well-informed layman, and would be useful to students. (C. N. H.)

The Molecular Basis of Neoplasia. Edited by Staff, University of Texas, M. D. Anderson Hospital and Tumor Institute. Austin: University of Texas Press, 1962 (614 pp., \$10.50).

This volume includes 35 papers presented at the Fifteenth Annual Symposium on Fundamental Cancer Research, 1961. Topics are divided into the following sections: Nucleic Acids, Nucleic Acids and Proteins, Protein Conformation and Sequence, Controlling Mechanism and Enzyme Synthesis, Controlling Mechanisms and Biochemical Alterations Induced by Viral Nucleic Acids, and Ribosomes and Protein Synthesis. Many of these papers are of interest to geneticists as well as biochemists. The individual papers are well-illustrated and include bibliographies, and the entire volume is indexed. (C. N. H.)

Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. Edited by Secretariat, United Nations. New York: United Nations Office of Conference Services, 1962. (442 pp., \$5.00, paper-bound).

The first report of this Committee was submitted in 1958. This second report places emphasis upon the new data appearing during the intervening four years, but is a self-contained document. The summary report of the 105-member Committee occupies only 35 pages, and the remainder of the volume is occupied by 11 "annexes" that summarize available information in specific areas. Annexes of particular interest to geneticists include those on the hereditary effects of radiation (34 pp.), somatic effects (89 pp.), medical and other exposures (39 pp.), and estimates of risks (7 pp.). The summary includes the following statements: "The concept of mutation induction as an instantaneous process has been revised and evidence accumulates showing that for some mutations a finite period of time elapses between the absorption of radiation energy and the completion of the mutation process, during which, depending on the physiological state of the cell, at least partial repair of the damage may be possible." "The frequency of gene mutations produced by irradiation has been shown to be proportional to the total dose received by the germ cells. The proportionality, however, has been shown, in mice, fruit flies and silk worms, to vary with certain factors including the dose rate." "... it is not possible to estimate with confidence a representative doubling dose for man." This report will undoubtedly be considered an authoritative statement on the topics discussed. (C. N. H.)

International Directory of Radioisotopes, Second Edition. By STAFF, INTERNATIONAL ATOMIC ENERGY AGENCY, Vienna. U. S. distributor, International Publications Inc., 801 Third Avenue, New York. 1962. (700 pp., \$9.00)

This cloth-bound single volume edition replaces the two-volume paperbound first edition of 1959. An international listing is given of all primary suppliers of radioactive compounds, various comments concerning facilities available in different countries, definitions of terms used, and an extensive table of all available compounds with suppliers and information concerning procurement. This directory will be of interest to those working with radioactive compounds. (C. N. H.)

Evolution and Illness: A Short Essay on the Clinical Significance of Evolutionary Vestiges. By R. M. J. HARPER. Edinburgh & London: E. & S. Livingstone Ltd. (U. S. agents: Williams and Wilkins Co., Baltimore), 1962. (108 pp., \$5.50)

This is a rather curious essay that presents an hypothesis concerning the importance of "evolutionary vestiges," particularly the pigmented nevus of the skin, the supernumerary nipple, and certain outmoded defense reflexes, in the etiology of certain diseases. In addition to emphasis upon skin conditions, there is discussion of childhood allergy, phlebotrombosis, peptic ulcer, diabetes mellitus, and neoplasia. The occurrence of evolutionary vestiges is attributed to persistence in some individuals of genes or gene complexes long since lost by most members of the species. The supporting evidence presented is not particularly impressive to this reviewer, but deserves consideration. (C. N. H.)

Growth at Adolescence. (Second Edition). By J. M. TANNER. Oxford: Blackwell Scientific Publications (U. S. Agents, F. A. Davis Company, Philadelphia 3): 1962. (325 pp., \$9.00).

A considerable amount of new material has been added to this new edition, particularly in the section on factors affecting the rate of growth and the age at puberty. Extensive discussions are provided of the adolescent child with respect to physical growth, physiological and endocrine changes, motor development, and changes in mentality and behavior. Numerous graphs are given in which various measurements are plotted against age. It is unfortunate that these give only average values without evidence of normal range or standard deviation. However, the source of all graphs and tables is given so that those interested may consult the original literature. The bibliography is extensive, and the book is well-indexed. (C. N. H.)

Birth to Maturity: A Study in Psychological Development. By JEROME KAGAN and HOWARD A. MOSS. New York: John Wiley & Sons, Inc., 1962. (381 pp., \$8.50)

The results of a longitudinal study of child psychology extending over a 30 year period at the Fels Research Institute are reported in this monograph. The findings of continuing studies of 71 children from birth to age 14 years are compared with a five hour battery of tests given to the same individuals as adults. The most consistent finding was that many of the behaviors exhibited by the child during the period of 6 to 10 years, and a few of those observed in the 3 to 6 year period, were moderately good predictors of theoretically related behaviors during young adulthood. The testing procedures used and the findings are described in detail, and the authors seem to be commendably cautious in drawing conclusions. (C. N. H.)

Neurosecretion. Edited by H. HELLER and R. B. CLARK. London and New York: Academic Press, 1962. (455 pp., \$14.50)

The third International Symposium on Neurosecretion was held at the University of Bristol in September, 1961. This volume includes the thirty-six papers presented with abstracts of discussion. Sections are devoted to the ultrastructure of neurosecretory systems, and to the histology and physiology of neurosecretory systems in vertebrates, annelids and arthropods. The text is well-illustrated with photographs and drawings, and well-indexed. (C. N. H.)

Primates: Comparative Anatomy and Taxonomy. Volume 5, Cebidae, Part B. By W. C. OSMAN HILL. Edinburgh: University Press; New York: Interscience Publishers, Division of John Wiley & Sons, 1962. (537 pp., 9:9/-).

The extremely detailed monographic series on comparative anatomy, taxonomy, and ecology of primates is continued in this fifth volume. The treatment of New World Primates is concluded in this section with the description of the sub-families Alouattinae and Atelinae. (C. N. H.)

Introductory Mycology (Second Edition). By CONSTANTINE JOHN ALEXOPOULOS. New York: John Wiley & Sons, 1962. 613 pp., \$12.00).

The Second Edition of this introductory textbook in mycology has been extensively revised. The approach is still primarily morphologic and taxonomic, but some physiological and genetic information is interspersed. Photographs and drawings are used extensively, and references for parallel reading appear at the end of each chapter. (C. N. H.)

Variation in Neonatal Death Rate and Birth Weight in the United States and Possible Relations to Environmental Radiation, Geology and Altitude

DOUGLAS GRAHN AND JACK KRATCHMAN

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, and Division of Plans and Reports, U. S. Atomic Energy Commission, Washington, D. C.

THE POTENTIAL BIOLOGICAL HAZARDS for man exposed to extremely low levels of ionizing radiation continue to be the subject of much debate. Ideally, the hazards are best evaluated in man himself, though there are many difficulties inherent in any survey or study of a human population. The United States population, for example, contains widely different social customs, economic levels, educational attainments, racial origins, and health practices. In addition, the age structure varies among the different regions and states, and a high degree of mobility is a population characteristic. In spite of these variables, there have been several attempts to evaluate the effects of environmental radiation on man, largely by means of the published vital statistics records. These have included the study of bone tumor incidence (Bugher and Mead, 1958), congenital malformations (Gentry, Parkhurst and Bulin, 1959; Kratchman and Grahn, 1959; Wesley, 1960), and leukemia incidence (Court Brown *et al.*, 1960; Craig and Seidman, 1961). The results of the tumor and leukemia incidence studies have all been negative. The malformation studies have been suggestive of a radiation effect, though alternative explanations and hidden biases were not entirely accounted for.

The present study is a more detailed follow-up of our previous preliminary report (Kratchman and Grahn, 1959). This study is not restricted to congenital malformation deaths, as these are not believed to be uniformly diagnosed in all regions. In addition, deaths from this cause are not presented in the vital statistics by age intervals. The calculation of comparative mortality rates would therefore require the age-standardization of all population groups. The neonatal death rate (deaths occurring within the first 28 days of life) is used instead, since it is normally based on the number of live births and therefore avoids the difficulties of a shifting population base. About 10 to 15 per cent of the neonatal deaths are attributed to malformations, and in addition, there is a sufficient variety of causes of death to permit the measure to reflect the effect of a number of intrinsic and extrinsic factors. The birth weight parameter was examined because of the high negative correlation that exists between it and the neonatal death rate.

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METHODS

Vital Statistics Data

The published vital statistics data of the U.S.A. (U. S. Department of Health, Education and Welfare) for the eight years 1950 through 1957 have been the source of all neonatal mortality and birth weight data, though the latter were not available for the years 1950 and 1951 in the form employed. The neonatal deaths and live births were tabulated by county of residence for the white population. The published listings are separated into "white" and "non-white" whenever 10 per cent of the population or 10,000 of their number are non-white. This category includes Negro, American Indian and Oriental races. When the 10 per cent or 10,000 criteria are not met, no separation is given, and therefore tabulations by county contain a small, but variable, proportion of non-white births and deaths. The sum effect of this is to raise the death rates a little above those published for the white population of a given state or region.

The birth weight data are published, by county, in two broad categories: "2,500 grams or less" and "2,501 grams or more." County tabulations for this study were re-expressed in terms of the percentage of births weighing 2,500 grams or less. Births of this category are classed as "immature," according to the Sixth Revision of the International Lists of Diseases and Causes of Death; therefore the weight parameter expresses the frequency of immature births in the population. The same breakdown of racial information pertains to both the weight and mortality data.

Several additional tabulations were made for selected states, regions, or years. These include: gestation length, mean birth weight, per cent born in hospitals, cause of death, and time of death. For the states of Delaware, Illinois, Indiana, Idaho and Montana, which contain virtually no uranium ore reserves, neonatal mortality rates were tabulated on a state-wide basis rather than by summation of individual counties for the purpose of rough comparison across an array of geologic environments.

Census Data

Certain characteristics of the surveyed population groups were tabulated to evaluate differences in age and socio-economic level. Median family income and median age values were tabulated, by county, from the published 1950 U. S. census data (U. S. Bureau of the Census, 1953). Where needed, other characteristics, such as median school years completed, per cent non-white, total population number, and population growth figures were also derived from the census reports.

Geologic Data

The geologic provinces of the U. S. were carefully defined in order that every county in the study could be assigned to a province. Where geologic borders did not coincide with political boundaries, assignment was made according to the location of the majority of the land area of the county.

Data as of January 1, 1962, on the location and magnitude of known ura-

nium ore reserves in terms of tons of U_3O_8 were obtained from the Division of Raw Materials, U. S. Atomic Energy Commission.

Altitude estimates were drawn from several sources, such as road maps and commercial atlases. However, most of the available figures, regardless of immediate source, are drawn from the accumulated data of the U. S. Geological Survey.

The mean county-altitude values finally employed are not mean values for the total physical topography, but are better classed as "mean populated altitude" figures. Individual locality elevations were multiplied (weighted) by the population number for the given locality. These were summed within the county and divided by the total population number to yield a weighted mean altitude. When county values are assembled into larger units, as states or provinces, the county altitudes were weighted by the number of live births. Thus, state altitude values are, for example, mean populated altitudes weighted by the number of live births at risk. The above procedure is critical for the present survey since mean physical elevations are nearly always well above the populated elevations.

Radiation Data

In the absence of detailed radiation dosimetry data, the preliminary report (Kratchman and Grahn, 1959), relied on the geographical distribution of uranium ore deposits as an indication of higher than average concentration of radioactive material in the natural environment. Although this presumption recognized the fact that most uranium reserves are highly localized, frequently deeply-buried, and in remote unpopulated areas, it was selected as a preliminary hypothesis on the assumption that an area containing large uranium deposits was presumed to be an environment which has a greater amount of disseminated uranium than an area which lacks deposits.

Since the publication of the preliminary report, additional studies have indicated that the assumption that uranium ore reserves are indicative of higher radiation levels may not be entirely appropriate. Nevertheless, there is a generally higher terrestrial radiation level in the mountain areas than in the mid-western region (Solon *et al.*, 1959). The average of 27 independent readings of environmental radiation (excluding terrestrial beta rays) is 11.7 microroentgens per hour or 103 milliroentgens per year for Ohio, Indiana, Illinois and Wisconsin. Of this, 66 mr is terrestrial, 37 cosmic. Forty-nine readings in Colorado, Wyoming, Utah and New Mexico averaged 20.2 μ r per hour or 177 mr per year. Of this, 104 mr is terrestrial and 73 cosmic. The terrestrial gamma radiation is therefore about 50 per cent and cosmic radiation nearly 100 per cent higher in the sampled regions of the mountain states.

These data of Solon *et al.* are the only direct measures available for the regions of interest and provide only a broad definition of the differences in radiation intensity. In view of the above remarks concerning the probable lack of close correlation between radiation levels and uranium reserves, Solon's data are probably sufficient for the question of external radiation levels. Radiation levels from the deposition of internal emitters have not been measured.

The dose rate from cosmic radiation was also measured and reported by Solon *et al.* (1959, 1960) for altitudes up to 17,000 feet after adjustment to a lati-

tude of about 41° N, which very nearly bisects the continental U. S. No attempt was made to correct for latitude variation since the cosmic flux rises only 14 per cent between 30° and 50° N at an altitude of 6,500 feet and less than 10 per cent at sea level. The altitude effect on radiation intensity is considerably greater and the majority of the populations studied reside in the more limited latitude range between 35° and 45° N.

Geographic Areas Selected for Study

Table 1 presents the number of counties in the study with and without known ore reserves by geologic province and state west of the Mississippi River. Fig. 1 outlines the geologic provinces in the U. S. and Fig. 2 indicates the states and counties included in the study. The map does not encompass the Texas Coastal Plain counties of Colorado, DeWitt, Fayette, Gonzales, Karnes and Lavaca.

Only portions of some states are included. These are:

- (1) California and Washington: Counties containing large granite batholiths and including several ore deposits.
- (2) North and South Dakota: Limited ore bearing areas with non-uranium areas for controls.
- (3) Nebraska: A non-uranium control area at moderate altitude.

TABLE 1. GEOLOGIC PROVINCES AND STATES INCLUDED
IN THE ANALYSIS OF NEONATAL MORTALITY RATES

Geologic Province	State	No. of counties	
		With U ₂ O ₈ reserves	Without U ₂ O ₈ reserves
Colorado Plateau	Arizona	3	0
	Colorado	6	5
	New Mexico	3	2
	Utah	7	7
Rocky Mountains	Colorado	9	18
	New Mexico	1	2
	Utah	0	5
	Washington	2	2
	Wyoming	4	9
Basin and Range	Arizona	3	8
	Nevada	4	13
	New Mexico	3	5
	Utah	2	8
Western Stable Region	Colorado	1	24
	Kansas	5 ^a	9 ^b
	Nebraska	0	11
	N. Dakota }	5	7
	S. Dakota }		
	New Mexico	0	16
	Texas	9 ^a	8 ^b
	Wyoming	5	5
Coastal Plain	Texas	6	7
Coast Ranges	California	2	9
		80	180

^aWith known helium reserves.

^bWithout known helium reserves.

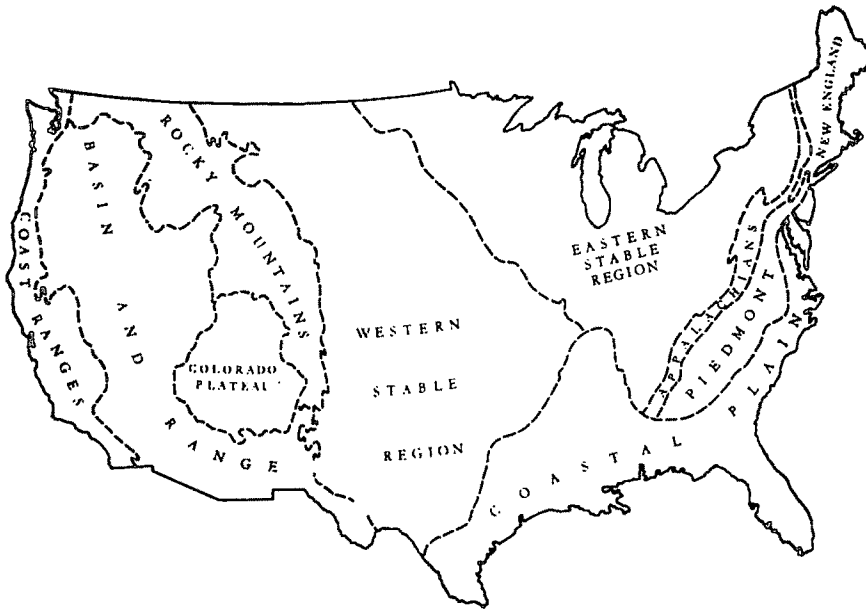


FIG. 1. Geologic provinces of the Continental United States.

- (4) Kansas and Texas Panhandle: Helium reserve area with non-helium areas as controls.
- (5) Texas Coast: Limited ore-bearing area with non-uranium areas as controls.

Control counties, in the specified instances, were chosen from geologic maps according to two criteria; contiguity with an ore bearing county and similarity of the geology.

RESULTS

Neonatal Mortality: Standardization of the Population

During the eight-year study period, a 15 per cent decline in neonatal mortality has occurred. The rate of change is essentially the same for the mountain states as for the whole U. S. (Fig. 3). This suggested that the time trend could be ignored and the data were pooled across the eight years.

An analysis of socio-economic factors was done for the years 1951-1954 for the nine geographic census divisions of the U. S. (these do not coincide with the geologic provinces and are defined in Volume II, Part 1, U. S. Summary, 1950 census). The eight states of the Mountain Division were also separately evaluated. The data for this analysis are given in table 2.

Several assumptions can be made concerning these measures of the socio-economic characteristics of the population: (a) median income reflects educational attainment and age, (b) neonatal mortality decreases as the probability of being born in a hospital increases and the latter is in turn positively related to income, (c) neonatal mortality decreases with increasing income. Income

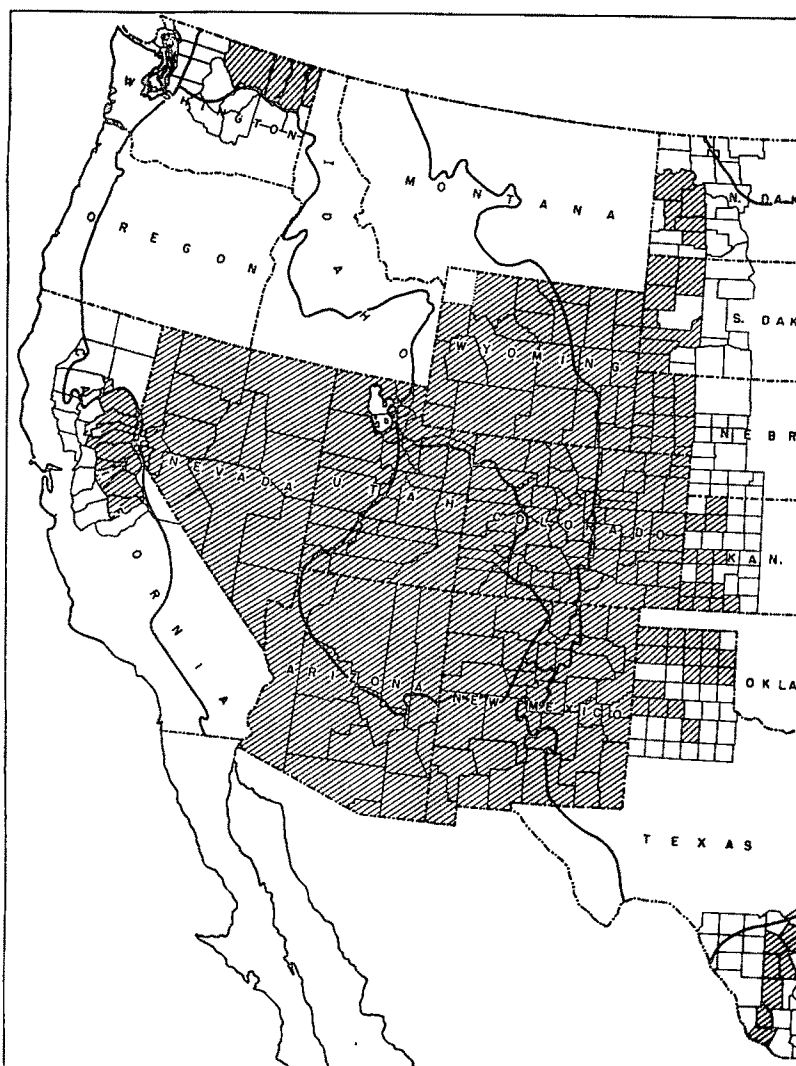


FIG. 2. Western portion of United States. Counties included in survey indicated by cross hatch. Heavy lines outline geologic provinces defined in Fig. 1.

therefore appears to characterize the general educational and social attainments and level of medical care in the population. Variation in median family income has consequently been used to account for the effect of differences in socio-economic level on neonatal mortality among the individual counties and states. The regression is $-2.0/1000$ live births per \$1,000 median income, and all county values were adjusted to a common income of \$3,000.

The relationship between maternal age and neonatal mortality is shown in Fig. 4. This is based upon data from a limited special study of live births that occurred during the first quarter of 1950 (U. S. Department of Health, Education and Welfare, 1958). Individual county mortality ratios were

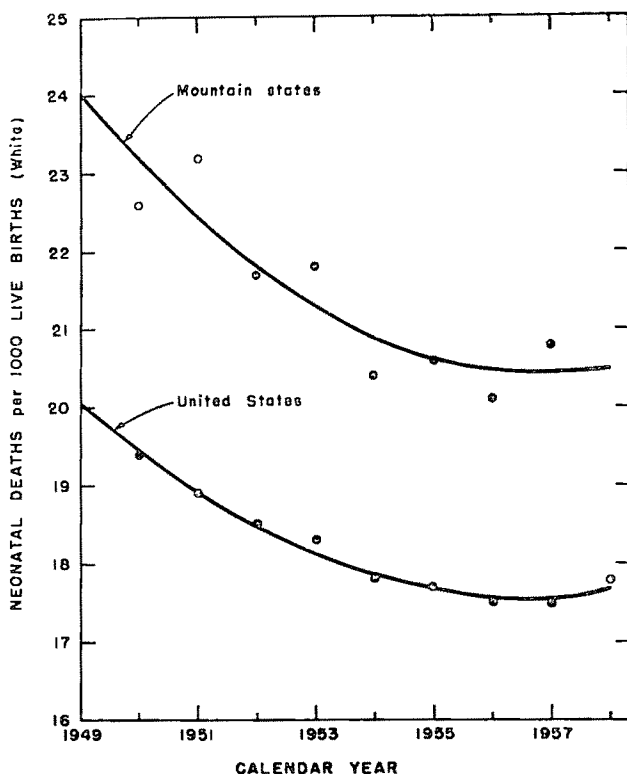


FIG. 3. Regression of neonatal death rate on time in years for U. S. and mountain states.

TABLE 2. SOCIO-ECONOMIC VALUES FOR U. S. CENSUS
DIVISIONS AND MOUNTAIN STATES

Division or State	Neonatal ^a Death Rate per 1,000	Median ^b School yrs.	Median Family ^b Income-Thous.	Median ^b Age yrs.	% Born in ^a Hospitals
New England	17.58	10.4	\$3.25	32.4	98.8
Middle Atlantic	17.43	9.5	3.40	33.0	98.3
E. North Central	17.85	9.7	3.43	31.4	98.1
W. North Central	17.88	9.1	2.90	31.2	97.0
South Atlantic	18.90	9.2	2.41	28.3	93.6
E. South Central	20.93	8.7	1.79	27.0	87.0
W. South Central	19.68	9.3	2.36	28.2	89.6
Mountain	20.98	10.9	3.10	27.8	95.1
Pacific	17.68	11.6	3.55	32.0	98.8
United States (Total)	18.37	9.7	3.07	30.8	95.9
Arizona	22.05	10.6	2.85	27.8	95.2
Colorado	22.40	10.9	3.07	29.6	96.2
Idaho	18.63	11.0	3.05	27.5	98.6
Montana	18.83	10.3	3.26	30.2	98.9
Nevada	20.80	11.7	3.61	32.1	99.4
New Mexico	25.03	9.5	2.65	24.4	82.1
Utah	16.63	12.0	3.26	25.0	98.9
Wyoming	22.25	11.1	3.48	28.0	98.9

^aBased on 1951-54 data for white population only.

^bFrom 1950 U. S. census data; income for 1949.

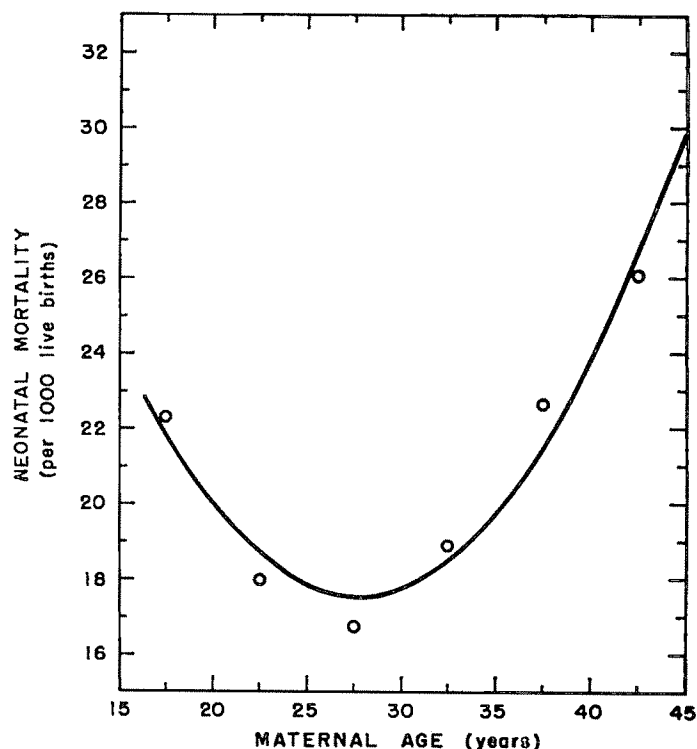


FIG. 4. Regression of neonatal death rate on maternal age, 1950 white population.

adjusted to a constant maternal age of 27.5 years by means of the equation: $Y = 49.8 - 2.33A + 0.042A^2$ where A is the maternal age. At 27.5 years, $Y = 17.5$, and is at a minimum value. For ages above or below 27.5, the mortality rate, Y_x , is always greater and the difference, $Y_x - 17.5$, is subtracted from the observed county value for adjustment purposes.

Comparison of States and Geologic Provinces

The age and income adjusted neonatal mortality figures for the individual states, or portions thereof, are given in table 3. Significant differences exist between Colorado, for example, and the Texas Coastal Plain counties, sampled regions of Washington and California, or the states of Delaware, Illinois and Indiana. The most striking difference involves the state of Utah, which has the lowest neonatal death rate in the U. S., significantly below its neighboring states. This is not associated with a high fetal death rate, so it apparently cannot be attributed to a classification difference for perinatal mortality. The individual county values in Utah are uniformly low but do vary from about 10/1000 to 24/1000, though this is a more limited range as compared to other states. A further analysis of the Utah data will be given below and it will be shown that Utah, though an exception, does adhere to certain basic relationships to be brought out. However, because of its unexplained deviation from other states, Utah is not included in subsequent analyses.

A comparison of geologic provinces (table 4) reveals differences that were already evident from the examination of their composite states. Significant differences do exist, but these are associated with the extremes of geographic location. The data in both tables 3 and 4 point to altitude as an important variable. However, ore reserves also are larger in the provinces and states at the higher altitudes where the higher mortality rates prevail, even though there appears to be no consistent effect that can be associated with the presence of uranium ore within the geologic provinces.

TABLE 3. AGE AND INCOME ADJUSTED NEONATAL DEATH RATES
AND ASSOCIATED PHYSICAL VARIABLES BY STATE; 1950-57
WHITE POPULATION ONLY

State	Altitude (feet)	U ₃ O ₈ Reserves (tons)	Atm. Press (mm Hg)	Cosmic rad. (mr/yr)	No. of Live Births	Neonatal Deaths ± SE (Per 1,000 births)
Texas (Coast)	310	570	752	35.1	37,216	18.13 ± 0.98
Washington	1,440	^a	721	41.9	11,078	18.01 ± 1.80
California	1,490	40	720	42.1	26,203	18.33 ± 1.17
Arizona	2,080	2,240	703	46.4	179,659	20.67 ± 0.47
Kansas	3,080	(Helium)	678	53.6	15,007	20.90 ± 1.65
Dakotas	3,180	2,680	676	54.2	33,975	20.10 ± 1.08
Texas (Panhandle)	3,370	(Helium)	672	55.3	35,192	21.26 ± 1.09
Nevada	3,690	190	664	58.0	40,360	22.64 ± 1.04
Nebraska	3,920	—	657	60.2	22,790	21.72 ± 1.37
New Mexico	5,010	80,330	632	69.6	189,242	23.41 ± 0.49
Colorado	5,390	11,670	623	73.1	306,818	22.19 ± 0.38
Wyoming	5,390	60,830	623	73.1	66,728	22.65 ± 0.82
Delaware	60	—	758	34.0	63,648	17.10 ± 0.73
Illinois and Indiana	630	—	744	36.8	2,285,385	17.40 ± 0.12
Idaho	3,320	—	673	55.1	131,306	18.25 ± 0.52
Montana	3,490	50	668	56.7	127,660	18.66 ± 0.54
Utah	4,590	12,190	642	65.6	191,042	16.63 ± 0.41

^aData not available for publication.

TABLE 4. AGE AND INCOME ADJUSTED NEONATAL
DEATH RATES BY GEOLOGIC PROVINCE

Geologic Province	U ₃ O ₈ Reserves (tons)	Altitude (feet)	No. of Live Births	Neonatal Death Rate ± SE
Basin and Range	670	3,550	74,180	20.96 ± 0.74
	Non-Ur.	2,700	213,699	21.89 ± 0.45
	Total	2,920	287,879	21.65 ± 0.38
Coastal Plain	570	330	20,024	18.74 ± 1.36
	Non-Ur.	280	17,192	17.43 ± 1.41
	Total	310	37,216	18.13 ± 0.98
Coast Ranges	40	2,390	2,371	19.51 ± 4.02
	Non-Ur.	1,400	23,832	18.21 ± 1.23
	Total	1,490	26,203	18.33 ± 1.17
Colorado Plateau	104,230	5,670	38,921	21.65 ± 1.05
	Non-Ur.	6,240	18,098	21.19 ± 1.52
	Total	5,850	57,019	21.51 ± 0.86
Rocky Mountains	62,580	5,440	66,864	21.30 ± 0.79
	Non-Ur.	6,100	59,827	21.56 ± 0.84
	Total	5,750	126,691	21.42 ± 0.58
Western Stable Region	2,700	4,320	37,127	21.04 ± 1.05
	Non-Ur.	4,790	341,934	22.81 ± 0.36
	Helium	3,220	27,508	21.04 ± 1.22
	Non-Helium	3,360	22,691	21.28 ± 1.36
	Total	4,570	429,260	22.46 ± 0.32

Altitude is not a simple variable. There is an increase in cosmic ray intensity, a decrease in oxygen partial pressure, an increase in ultraviolet radiation, and a decrease in average humidity and temperature accompanying altitude increase. Although a search for radiation effects underlies this study, oxygen tension is one environmental factor that cannot easily be dismissed, since it has long been recognized as a factor in the disturbed reproductive physiology seen at high altitudes (Monge, 1948). Fig. 5 presents the mortality data from table 3 plotted against cosmic ray intensity and atmospheric pressure in terms of mm of mercury. The latter can be re-expressed as the partial pressure of oxygen by multiplying the abscissal values by 0.2096. The equations for the two relationships are:

$$Y_1 = 13.42 + (0.1343 \pm 0.0205)D \text{ and} \\ Y_2 = 48.92 - (0.415 \pm 0.0058)P$$

where Y_1 and Y_2 are the predicted neonatal death rates per 1,000 live births, D is annual cosmic ray dose in milliroentgens, and P is atmospheric pressure in millimeters of mercury. Both regressions are highly significant ($r_{Y,D} = +0.900$; $r_{Y,P} = -0.914$) and the regression on atmospheric pressure is slightly, but not significantly, the better fit. The values of Y at sea level are:

$$Y_1 = 17.89, \quad D = 33.3 \text{ mr} \\ Y_2 = 17.38, \quad P = 760 \text{ mm}$$

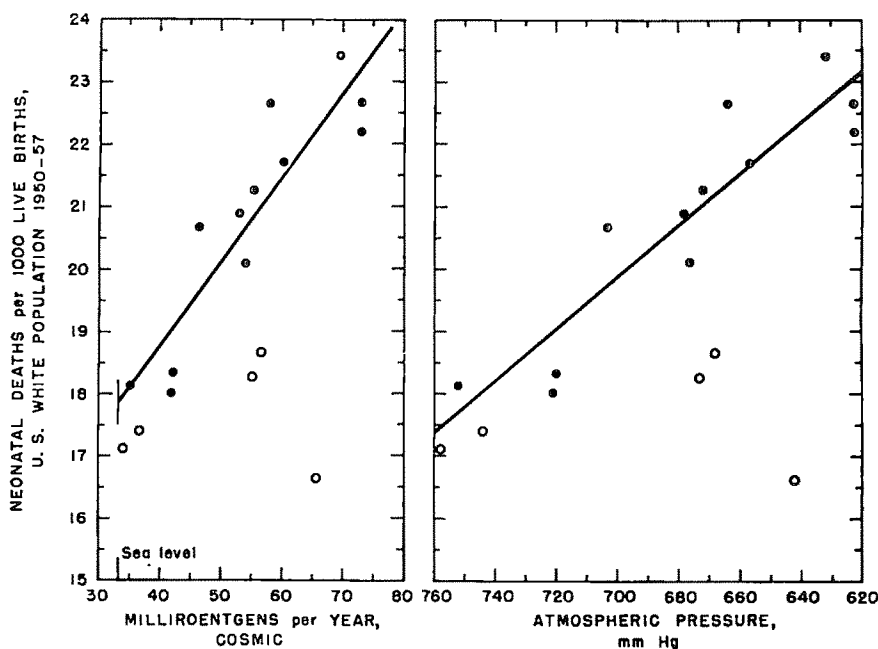


FIG. 5. Regressions of age and income-adjusted neonatal death rate on annual cosmic radiation dose and atmospheric pressure. Open circles not included in calculation (see text and table 3). To facilitate comparison, values for atmospheric pressure are plotted from high to low.

The regression of mortality rate (Y_3) on altitude in feet (A) is

$$Y_3 = 17.51 + (1.04 \times 10^{-3} \pm 0.15 \times 10^{-3})A.$$

This regression fits the data about as well as that for atmospheric pressure and is used for certain data adjustments and comparisons. It should be noted that neither cosmic radiation intensity nor atmospheric pressure is linearly related to altitude.

In all three cases, the regression coefficient is the least squares estimate from the data in the upper portion of table 3. Data for the states of Delaware, Illinois, Indiana, Idaho and Montana are not employed since, as noted previously, these data are not summarized by county and therefore do not include the small portion of non-white births and deaths which invariably elevate the final mortality rates. Utah is the exception, but within the state the data are consistent with the relationships noted in Fig. 5. Individual counties in Utah range from 3,500 to 7,000 feet and a progressive increase in mortality rate is detectable across this range. The rate of change is similar to the above noted regressions and the equation for Utah is

$$Y = 7.78 + (1.83 \times 10^{-3} \pm 0.79 \times 10^{-3})A.$$

Comparison of Areas with and without Uranium Ore

The data in table 4 also present the mortality rates in the six provinces according to the presence or absence of uranium ore and, for western Kansas and the Texas Panhandle, the presence or absence of helium reserves. The helium reserves are considered since, as Kratchman and Grahn (1959) indicated, these reserves may be the result of the disintegration of localized disseminations of uranium and therefore reflect a higher than average level of environmental radiation. The differences within provinces are not significant and uranium-bearing counties are as likely to have mortality rates above as below their control areas. There is, however, a small confounding of altitude in these comparisons.

In order to ascertain if any relationship may exist between mortality and the presence of uranium ore in the absence of the altitude variable, the 57 counties with ore reserves (excluding Utah) were adjusted to a constant altitude of 3,000 feet. These adjusted mortality rates were plotted against a crude measure of ore concentration; tons of U_3O_8 per 1,000 square miles. No correlation was evident even across six log cycles of difference in ore concentration. The regression coefficient of death rate on log ore concentration is $+0.113 \pm 0.625$. *Thus, it can be concluded that the quantity of uranium ore reserves is unassociated with the probability of neonatal mortality.* However, in the absence of direct measurement of radiation levels in the 57 counties, these results cannot be considered as having entirely eliminated an interpretation based on radiation-induced injury.

Birth Weight and Associated Parameters

In the course of the analysis of neonatal mortality, it became increasingly apparent that other measures associated with the birth event had to be examined,

since the observed relationship with altitude strongly suggested the existence of an underlying subtle disturbance of reproductive physiology.

Table 5 presents data drawn from the 1956 and 1958 statistics on gestation length and birth weight for the indicated states that were selected for their distribution between sea level and 6,000 feet and the adequacy of sample size for live births. There is a decline in the duration of gestation with increasing altitude, but the maximum difference is only about 1 per cent of the average duration, or about 0.4 weeks. This is not significant and will not explain the birth weight difference of 190 grams between Colorado and Illinois-Indiana. Fig. 6 describes the fetal growth curves for Illinois-Indiana versus Colorado-New Mexico for the years 1950-51. This suggests that during the last 10 weeks of gestation the growth rate of the fetus at 5,000 feet or more progressively falls behind that for the fetus at or near sea level.

TABLE 5. DURATION OF GESTATION AND BIRTH WEIGHT FOR SELECTED STATES; 1956 AND 1958 WHITE POPULATION ONLY

State	Altitude (feet)	Duration of gestation (wks)	Birth weight (grams)
Illinois-Indiana	630	40.40	3,342
Arizona	2,080	40.28	3,300
Idaho-Montana	3,400	40.13	3,290
Utah	4,590	40.23	3,288
New Mexico	5,010	40.17	3,169
Colorado	5,390	40.01	3,152

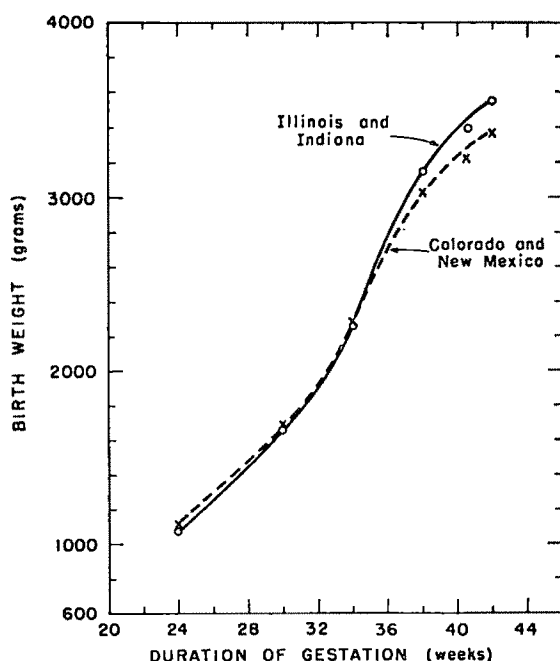


FIG. 6. Fetal growth curves derived from data on live birth weights for 1950-51 white population.

Age-Specific Mortality and Causes of Death

The time and causes of death have been examined for several selected states with the hope this might reveal differences that could assist in the interpretation of the mortality and birth weight data. It should be emphasized, however, that the cause of death data are subject to considerable difference of diagnostic opinion and can only be evaluated in a broad sense.

Table 7 gives the death rate, by specific cause, for the total neonatal period, of Illinois-Indiana and Colorado-Wyoming. This comparison is emphasized on the assumption that these four states would be more similar in their levels of medical practice and socio-economic status than comparisons involving the border states. About 95 per cent of all deaths are included in the selected categories.

Most of the deaths fall into a few classes: malformations, birth injuries, post-natal asphyxia and atelectasis, and immaturity. The death rate from birth injuries and immaturity is 30 to 50 per cent higher in Colorado-Wyoming than in Illinois-Indiana, while only slight increases are noted for malformations and asphyxia. Other causes vary in the degree of excess, but a few points bear mention. Several causes of death are predominantly of genetic origin; congenital malformation, erythroblastosis, hernia and intestinal obstructions, and possibly asphyxia and atelectasis. These causes are elevated in frequency by only 2 to 18 per cent in the mountain states, while the other causes are elevated by 35 to 137 per cent. Since birth weight is lower at higher altitudes, it is not surprising to find an excess of mortality associated with immaturity as a concurrent qualification or as an unqualified cause.

TABLE 7. MORTALITY RATIOS FOR SELECTED CAUSES OF DEATH;
1950-57 WHITE POPULATION ONLY. ILLINOIS-INDIANA = 1.00

Cause of Death	Ill.-Ind. Death Rate (per 10,000 live births)	Mortality Ratio		
		Colorado- Wyoming	New Mexico	Utah
Hernia; intest. obst.	1.73	1.18	1.14	0.92
Cong. malformation	25.91	1.02	1.05	0.98
Birth injury	26.08	1.48	1.21	0.89
w/o immat.	11.28	1.12	1.24	0.86
with immat.	14.80	1.76	1.19	0.91
Postnatal asphyxia and atelectasis	47.80	1.07	0.81	0.80
w/o immat.	13.92	0.81	0.96	0.79
with immat.	33.88	1.17	0.75	0.81
Pneumonia	6.66	1.44	1.43	0.51
w/o immat.	4.55	1.26	1.48	0.47
with immat.	2.11	1.84	1.35	0.60
Diarrhea	0.88	2.19	5.50	0.72
Matern. toxemia	1.24	1.59	1.59	1.80
Erythroblastosis	6.97	1.06	1.12	1.23
Hemorrhagic dis.	1.73	1.64	0.95	1.19
Ill defined dis.	3.55	2.37	3.89	1.69
w/o immat.	0.66	1.51	4.05	1.60
with immat.	2.89	2.57	3.86	1.71
Immaturity	43.63	1.35	1.81	1.03
Accidents	1.07	1.65	2.59	0.84
All causes	173.03	1.28	1.40	0.95

Data for New Mexico and Utah are also presented in table 7. Although Utah has a total death-rate ratio to Illinois-Indiana of 0.95, there is an array of causes of death that are in excess. These include maternal toxemia, erythroblastosis, hemorrhagic diseases and the ill-defined diseases (largely nutritional maladjustment and congenital debility).

While variation in diagnostic accuracy and completeness certainly exists, immaturity and the ill-defined diseases are definitely excessive as causes of death in New Mexico, Utah, Colorado, and Wyoming. Except for erythroblastosis in Utah, the causes that may be more genetic in origin are not particularly in excess in these states.

During the first 28 days of life, the death rate drops almost exponentially. The mountain states, exclusive of Utah, have a higher rate of mortality throughout the whole time period, but it is not uniformly in excess of the Illinois-Indiana base line. The data in table 8 and Fig. 8 indicate the existence of a sharp increase in the mortality ratio for Colorado-Wyoming during the second and third full days of life. This drops back rapidly in the latter half of the first week, then rises again to a more steady level of excess mortality during the second through fourth weeks of life. This plateau continues throughout most of the first year. The early peak also appears in the data of New Mexico and Arizona though less markedly for Arizona. Since the latter has a mean altitude of about 2,000 feet compared to the 5,000 feet or more in the other states, this early period of high risk may be a characteristic of high altitude populations. Unfortunately, the published data are not reported in a way that permits the calculation of age-specific mortality rates by cause for the individual states, so it is not possible to associate the early period of high risk with specific causes.

The mortality ratios for the state of Utah generally conform to the results for the surrounding mountain states. There is a higher level of mortality during the first week, though it tends to broaden out over most of the week before dropping to a lower stable level. Since the Utah data demonstrate a small mortality excess due to immaturity, the early peak may be the result of a more rapid selection against the immature infant when subject to the additional stress of a lower ambient partial pressure of oxygen.

TABLE 8. MORTALITY RATIOS FOR ALL CAUSES OF DEATH
DURING NEONATAL PERIOD; 1950-57 WHITE POPULATION
ONLY. ILLINOIS-INDIANA = 1.00

Age (days)	Ill.-Ind. Death Rate (per 10,000 per day)	Mortality Ratio			
		Colo.- Wyo.	New Mexico	Ariz.	Utah
0	927	1.20	1.26	1.13	0.81
1	250	1.46	1.38	1.28	1.41
2	171	1.27	1.60	1.29	1.00
3	90	1.16	1.42	1.24	1.00
4	51	1.08	1.43	1.20	1.28
5	37	1.19	1.41	1.19	1.00
6	28	1.14	1.32	1.29	0.89
7-13	13.7	1.32	1.80	1.48	0.88
14-20	7.7	1.34	2.17	1.38	0.89
21-27	5.4	1.37	2.38	1.60	0.92

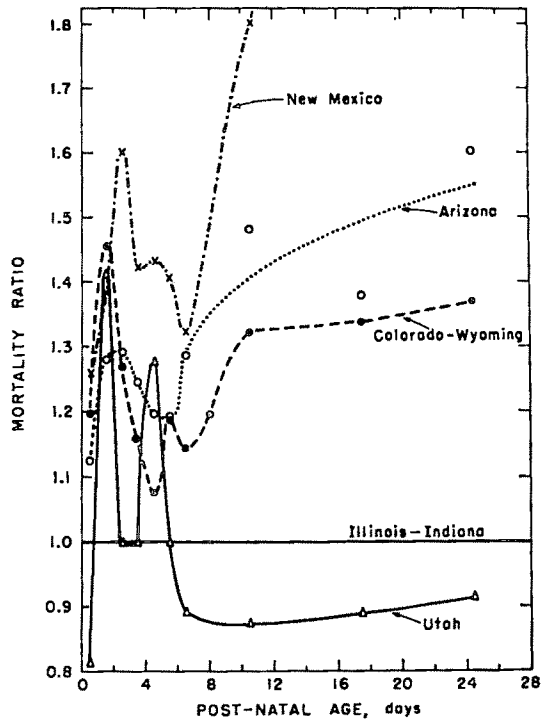


FIG. 8. Mortality ratios for age-specific mortality from all causes. Ratio equals New Mexico/Illinois-Indiana, etc.

DISCUSSION

The neonatal mortality rate is unquestionably higher in most of the mountain states than in the coastal and midwestern regions. The analysis clearly indicates that there is a regular increase in neonatal death rate with increasing altitude and that this relationship is probably independent of the geologic environment. The correlation with altitude, however, may be due to a number of factors, but the associated increase in cosmic ray intensity and decrease in oxygen partial pressure would appear to require special attention.

For the most part, the increased neonatal death rate appears to be a direct function of the lower birth weight that characterizes the higher altitude populations. The proportion of live births classed as immature progressively increases with altitude, and there is evidence that this may be due to a depression of fetal growth. In addition, the age-specific mortality rates indicate the existence of a peak of the mortality-ratio in the first days of life for the infants at higher altitude compared to Illinois and Indiana.

Radiation as a Causative Agent

The original working hypothesis of this study, that increased levels of environmental radiation underlie the higher neonatal mortality rates, is based on genetic concepts. Specifically, it was assumed that higher radiation levels would result in an increased mutation rate that would express itself in part by an increase in

early mortality. However, it is extremely difficult either to prove or disprove a genetic argument on the basis of only the published vital statistics data.

Although neonatal mortality is known to have a genetic component (see summary by Stern, 1960), different studies vary in the importance accorded the genotype for the control of perinatal and neonatal deaths. There is apparently no simple way of calculating the proportion of genetic deaths occurring in the neonatal period; we roughly estimate the figure to be about 20 per cent. This assumes that all of the erythroblastosis, one-half of the hernia and malformation, one-fifth of the atelectasis and one-tenth of the pneumonia and immature categories are genetic in origin. Most of these deaths are in the erythroblastosis and congenital malformation categories. Examination of the data revealed that the mortality rates for these two causes of death are very similar in the different parts of the United States that were surveyed. This is particularly true for malformation deaths, which would suggest that a genetic hypothesis to explain the higher neonatal death rates is not appropriate.

Data of the present type have sometimes been employed for the calculation of the genetic doubling dose for man. Such efforts are not appropriate. A brief exploration of the concept will emphasize this and indicate the tenuous nature of any radiation genetic hypothesis to interpret these or similar data reported by Gentry *et al.* (1959) and Wesley (1960). If a doubling dose were to be calculated, two assumptions concerning the population are implicit. One, it is assumed that the population is in genetic equilibrium with respect to the induction and elimination of detrimental genes. Two, it is further assumed that all excess mortality can be attributed to genetic factors. Neither of these assumptions can be met. If one now assumes that excess neonatal death is due to dominant genes, only a single generation would be required to reach equilibrium. However, if this were the case, the Hiroshima-Nagasaki survivorship should have demonstrated tremendous increases in the incidence of early mortality, whereas the actual changes were either negative or insignificantly positive (Neel and Schull, 1956). The average dose to the exposed Japanese population was between 35 to 40 r while that to the U. S. mountain population is only about 2 r/generation/gamete above the sea level exposure.

Certainly, the U. S. population is not in genetic equilibrium. In most of the mountain regions, there have been only about three generations at risk. The average generation number would be less, since these populations have grown by a factor of about 70 in the last 100 years while the balance of the U. S. has grown by a factor of only 6 or 7. Most of this difference must be due to migration, which reduces the rate of approach to equilibrium. Therefore, if one did then assume the population to be in equilibrium for recessive genes, the probability of death of the heterozygote would have to be over 30 per cent, which is about 10 times greater than generally noted (Morton, Crow and Muller, 1956; Fraser, 1962).

Birth weight also has a genetic component (Morton, 1955, 1958; Penrose, 1954), but the amount of genetic variation appears small. If weight is controlled by a polymorphic genetic system where weights in the 2,500 to 4,000 gram interval represent the adaptive norm, as suggested by Stern (1960), then

the observed changes in birth weight distribution are contrary to genetic expectation. In this study, the whole distribution of weights is shifted, rather than an increase occurring in only the extreme weight classes, as would be expected if there were an increased segregation of detrimental homozygous gene combinations. A polygenic, additive genetic system under the pressure of an increased frequency of detrimental genes would behave in the manner observed, but again, the 190-gram weight shift is far in excess of any reasonable expectation according to experience (Neel and Schull, 1956; Morton, 1958).

Direct irradiation of the fetus must also be considered as a possible basis for the decreased birth weight and increased mortality rate. In the mountain states, the fetus would receive a dose of 50 to 60 mr over that at sea level, and protracted over the full gestation period. There are no comparative experimental or clinical data at this extremely low dose rate or total dose. There are some data, however, on mice (Russell, Badgett and Saylor, 1960) and rats (Brown *et al.*, 1962) exposed at rates between 2 r and 20 r/day. At doses of 10 r/day or less there is no significant effect on birth weight.

Microcephaly and other deformities have been observed among children exposed *in utero* at Hiroshima and Nagasaki (Plummer, 1952). These were all among the more heavily irradiated as judged by radiation injury and distance from the hypocenter. Doses of several hundred roentgens or more were probably involved. A search for abnormal skeletal development among children irradiated *in utero* was negative (Sutow and West, 1955). Birth weight data are not available on these children.

The observed weight and mortality parameters in the U. S. mountain states are similar to those of radiation-induced injury from exposure *in utero*, but, as with genetic considerations, the present data indicate changes far in excess of any clinical or experimental radiation experience. If radiation were the predominant causative agent through either genetic or somatic pathways, man would be characterized by such an extremely high level of radiosensitivity that it would almost certainly have been detected many years ago, and particularly in the Japanese studies. As the data stand, there is no evidence for the existence of such an extreme radiosensitivity.

Hypoxia as a Causative Agent

It has been mentioned above that a reduced partial pressure of oxygen has a detrimental effect on reproductive physiology. A fascinating report by Monge (1948) portrayed the historical and even evolutionary significance of this problem in the Spanish colonization of Peru. For example, the original Spanish capital city, Jauja, situated at 10,800 feet, was moved to the present location, Lima, near sea level, because of the relative infertility and high neonatal death rate among the livestock.

Reduced viability and an increased probability of early death has been observed in domestic and laboratory animals, as well as in man, and the problem is of some economic importance for poultry breeding in the U. S. mountain states. Smith and Abbott (1961) noted that White Leghorn chickens have only 3 per cent hatchability at 10,150 feet, although 93 per cent of the eggs are

fertile. Sixty per cent hatch at sea level, and eggs laid at high altitude but brought to sea level for incubation have a normal hatch rate. Thus, the effect of altitude is transient. Additional evidence of the importance of oxygen was given by Davis (1955) and Moreng and Hartung (1959) who noted the improvement of hatch rate when the incubator air is supplemented with oxygen.

In the Sprague-Dawley rat, exposure to a simulated altitude of 18,000 feet for four hours a day reduces the average litter size by over 40 per cent, and survival between birth and 21 days of age drops from 90 per cent to 60 to 70 per cent (Altland, 1949). Most of the drop in litter size was attributed to fetal resorption. The only persistently noted lesion was marginal necrosis and hemorrhage of the placenta, which was attributed to hypoxia. An additional report by Chiodi (1953) indicated that 35 per cent mortality occurred in the first three days of life among rats born at 12,000 feet. This is prevented by raising the oxygen tension to sea level values.

The most significant data on man were derived from a study of infants born in Lake County, Colorado (10,000 + feet), which is the highest county in the U. S. Lake County infants are 380 grams lighter than Denver infants (Lichty *et al.*, 1957) but congenital defects were no more frequent there than in Denver or New York. There was no effect attributable to race, socio-economic status or diet. Body length and head size were also smaller, but in accordance with normal relations between weight and length or circumference (Howard, Lichty and Bruns, 1957). Thus the reduced birth weight was due to an over-all reduction of growth. These clinical measures conform to the earlier statistical observation that the whole distribution of birth weights is shifted to lower values.

One additional observation in the study by Howard *et al.* is particularly significant. This relates to the infants of mothers who had previously borne children outside of Lake County. For 120 mothers who met this criterion, there were 293 prior children with a mean birth weight of 3,130 grams and 261 children born in Lake County with an average weight of 2,840 grams. The 290-gram difference is more striking when considering that mean birth weight normally rises slightly with increasing maternal age. This observation emphasizes the direct nature of the altitude effect on the maternal-fetal physiological relationships.

The exact physiologic mechanism of the effect of reduced oxygen tension on fetal growth and neonatal mortality is somewhat obscure. An attempt to synthesize the observations and considerations of a number of investigators does offer the following as a possible interpretation (Barcroft, 1938; Boell, 1955; Windle, 1941; Acheson, Dawes and Mott, 1957; Metcalfe *et al.*, 1962). During the period of major differentiation and organogenesis, the oxygen supply is more than adequate as growth of the placenta is in excess of the demand placed upon it. The placenta, however, is structurally limited and therefore the blood volume it can handle is ultimately limited. Although minor fluctuations in oxygen exchange normally occur, there appear to be effective compensating mechanisms for this. When the available oxygen is limited by the physical environment, however, compensation by the fetus will take the form of a reduced

oxygen requirement and the amount available for growth must therefore be reduced. In this way, average fetal growth rate in the last trimester could easily be depressed at higher altitudes, where the maternal and uterine environments are unable to provide the normal O₂ requirements.

Since the altitudes in the U. S. are not exceptionally high and the fetal death rates in the mountain states are not excessive, it can be concluded that the lower birth weight and subsequently increased neonatal death rate are an expression of minor adaptive failures detectable only in large populations.

The study has thus touched upon some interesting considerations of human ecology and the adaptive capabilities of man. Neonatal death might well be considered an extended expression of "pregnancy wastage," and of the most expensive form, both biologically and economically. The mountain states have a 20 per cent to 30 per cent higher rate of wastage than the U. S. averages but the pregnancy losses appear to be more than compensated by a greater reproductive activity. The preliminary reports of the 1960 census (U. S. Bureau of the Census, 1961) presented a measure of reproductive performance, the "fertility ratio," for all U. S. regions and states. This ratio is the number of children under 5 years of age per 1,000 women between the ages 15 and 49. The mountain region has the highest fertility ratio in the U. S.; 560, compared to the U. S. average of 488. There is also a generally positive relationship between this ratio and the neonatal death rate across the whole U. S.

Very likely, the non-transient residents of the mountain states become progressively acclimatized and less susceptible to pregnancy loss. However, early death is also a very effective means of natural selection for parent stock of greater adaptability. The existence of what would now be recognized as a genetic basis for resistance to hypoxia was described centuries ago by the Spanish colonists in Peru (Monge, 1948). Inter-marriage of Peruvian Indian and Spanish produced offspring more capable of survival at high altitude. It was noted that infants with one parent of pure Indian descent enjoyed greater viability than those with one-quarter, one-eighth or less admixtures of the native genotype. Selection for fertile and fecund breeding stock in sheep has also been successful in the Andes. In this country, selection experiments with poultry have succeeded in improving the viability of standard breeds at elevations above 7,000 feet (Davis, 1955; Smith and Abbott, 1961). Heritability of resistance to the hypoxic environment has been estimated to be between 0.30 and 0.65, comparable to other estimates of heritability of viability in poultry (Lerner, 1950). Thus, the high-altitude environment is an excellent example of a general environmental stress factor to which man and the domestic animals can or have responded in the most classical manner, by selection for the viable genotype.

In conclusion, a word should be said about previous efforts to relate congenital malformation frequency and death rate to environmental radiation. The study by Wesley (1960) hardly deserves mention, since it was inappropriate to accept world-wide figures on malformation deaths as diagnostically accurate and comparable. The study by Gentry *et al.* (1959) was carefully done and relatively complete. Although New York state has some areas with altitudes

above 3,000 feet, as in the Adirondacks, the results probably cannot be attributed to altitude, since altitude appears to have little influence on malformation incidence, even though brief periods of severe anoxia are known to induce malformations in mice (Ingalls, Curley and Prindle, 1952). It is difficult to interpret the New York study, but the present study certainly would not support a radiation genetic hypothesis, as put forth by Gentry *et al.* The completeness of ascertainment of malformations is open to question, since the original study detected an under-reporting that may have been as high as 50 per cent. Urban-rural differences in the continuity of medical observation may also be a problem. Lastly, in spite of the existence in the mountain states of local uranium concentrations many times greater than in New York, no detrimental effects could be attributed solely to the geologic environment.

SUMMARY

Variation in the neonatal death rate in selected areas of the Western United States has been evaluated with reference to the geologic environment and the presence or absence of known uranium and helium reserves. While the neonatal death rate is unquestionably higher in the mountain regions, this does not appear to be attributable to higher levels of terrestrial radiation. A significant positive relationship does exist between death rate and altitude. The increased death rate can be largely attributed to a lower birth weight, since the frequency of immature births, on a weight criterion, progressively increases with altitude. The observed correlations with altitude have been evaluated in terms of either the increase in cosmic radiation intensity or the decrease in oxygen partial pressure, or both. The data have been evaluated in terms of three hypotheses; radiation-induced mutation, radiation-induced injury to the fetus, and hypoxia-induced depression of fetal growth. Very little of the excess neonatal death can be attributed to the readily-defined genetic factors, and direct fetal irradiation was concluded to be of no significance. The weight of the evidence—historical, experimental, and clinical—strongly suggests that the reduced partial pressure of oxygen is responsible for the reduced fetal growth and subsequently increased neonatal death rate.

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Determination of Phenotypes in the Human Group-Specific Component (Gc) System by Starch Gel Electrophoresis

W. CAREY PARKER, HARTWIG CLEVE AND ALEXANDER G. BEARN

The Rockefeller Institute, New York, New York

THE TECHNIQUE of starch gel electrophoresis (Smithies, 1955; 1959a) as an analytical method for the resolution of complex mixtures of high molecular weight substances has received extensive application in the study of numerous enzyme and protein systems in human and animal species. By means of specific staining techniques and the use of radioactive markers, it has been possible to visualize individual protein components in the gel. Application of the starch gel technique to the study of human serum proteins enabled Smithies (1955, 1957) to detect hereditary variations in haptoglobin, the α_2 -hemoglobin-binding globulin, and in transferrin, the β_1 -iron-binding globulin (Giblett, 1962). Resolution of the multiple components in the human transferrin polymorphism was facilitated by electrophoresis under conditions of high voltage and low temperature (Parker and Bearn, 1962), and in the present report a similar technique is described which permits application of starch gel electrophoresis to the determination of phenotypes in another human serum protein polymorphism, the Gc (Group-specific component) system. Inherited variations in Gc were originally detected by the method of immunoelectrophoresis (Hirschfeld, 1959b), and the results of various investigations in this field have been reviewed by Cleve and Bearn (1962). In the present report, conditions of electrophoresis are defined for the resolution of the two common components of the Gc system, and the usefulness of the method is illustrated by the identification of three rare Gc variants. In the adjoining report (Cleve *et al.*, 1963) results are presented of an extensive genetic investigation of two of these variants (*GcChippewa* and *GcAborigine*). A technique is also described which permits the rapid and routine analysis of 90 samples in a single starch gel, which can be sliced into several layers for the application of appropriate staining techniques.

MATERIALS AND METHODS

Starch: In the course of these investigations, three lots of commercially available starch (Starch Hydrolyzed; Connaught Laboratories, Toronto) were used; with Lot Nos. 165-1 and 176-1, optimum resolution of the Gc proteins was obtained; with Lot 173-1, less satisfactory resolution was observed which appeared to result from the reduced specific advancement of albumin at the

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interface between the gel and the plastic (lucite) supporting mold. This phenomenon is described in detail in the discussion.

The optimum concentration of starch in the gel was 13.0% w/v (Lot 165-1 recommended 12.0% and Lot 176-1, 12.5%). All gels illustrated in the present study were prepared at this concentration. Experiments were carried out to investigate the resolution of the Gc proteins at various starch concentrations from 11 to 15 per cent. At 12.5% starch, the trailing albumin border was slightly more blurred, and the Gc proteins were not as sharply defined as at 13%, although there was a greater separation between Gc 1 and the trailing border of albumin. At 13.5%, conversely, the trailing albumin border was sharper, and Gc 1 migrated closer to this border; similarly, the Gc proteins were not as clear. At 12% starch concentration the effects observed at 12.5% were more accentuated, and at 11% the post-albumins were so blurred that the Gc phenotype could not be distinguished; correspondingly, at 14% starch, the effects observed at 13.5% were accentuated, and at 15% the Gc 1 protein migrated at the trailing albumin border.

Buffer: In all experiments the starch gel was made up in a borate buffer of low ionic strength (.020M H_3BO_3 , .0084M NaOH; pH 8.92); a more concentrated buffer (.30M H_3BO_3 , .060M NaOH; pH 8.2) was used in the electrode vessels. The more concentrated buffers which were recommended with each lot of Starch Hydrolysed provided less satisfactory resolution of proteins in the post-albumin region as did the tris-borate discontinuous buffer system (Poulik, 1957). By horizontal starch gel electrophoresis in the tris-borate buffer, Schultze *et al.* (1962a; 1962b) have localized a purified Gc 2-1 preparation to the post-albumin region and have shown a clear resolution between Gc 1 and Gc 2 in this sample. These authors also demonstrated the resolution of Gc 1 and Gc 2 in whole serum. However, experiments carried out during the present study have not been able to achieve the necessary reproducibility in the horizontal tris-borate system to permit routine Gc classification of whole serum.

Preparation of Gels: Since the determination of Gc 1 depends upon the relatively enhanced mobility of albumin at the gel-lucite interface, experiments were carried out in an attempt to modify this interface. In routine experiments, a thin layer of mineral oil (Squibb 5592) was spread on the Lucite mold to ease removal of the gel from the mold after electrophoresis. Replacement of the mineral oil with a layer of petroleum jelly (Vaseline), cellophane film (Saran Wrap), or Parafilm caused no change in the observed Gc pattern. Rapid cooling of the hot starch solution in the lucite mold by contact between the bottom of the mold and crushed dry ice did not perceptibly alter the Gc pattern. Slow cooling of the starch in the mold by the circulation of water at 65° C through the bottom of the mold also produced no effect on the Gc separation. No difference in the Gc pattern was observed by normal cooling of the gel at room temperature for periods of 12-24 hours, although after long periods of cooling, the pattern became slightly more blurred.

Conditions of Electrophoresis: Optimum Gc separation was achieved in these experiments by electrophoresis in the vertical starch gel system of Smithies

(1959a) for 3-4 hours at 20 volts/cm and 4° C in gels of 6 mm thickness. During electrophoresis, two electric fans (Hunter-RM model FO12, 1000 CFM) were placed at the sides of each gel, and the gels were air-cooled throughout the run. To facilitate heat exchange, gels were covered with cellophane film instead of petroleum jelly. Water-cooling of the gel molds during electrophoresis was also employed, although it was unnecessary for resolution of the Gc proteins. Additional experiments were carried out with gels of 3 mm thickness at voltage gradients up to 33 volts/cm without improving the resolution of either the Gc pattern or the overall serum protein pattern.

Population Screening: For the survey of large numbers of samples, it has been convenient to use 30-slot inserts (tiers) in conventional (30 x 12 x 0.6 cm) vertical starch gel electrophoresis molds. Each filled slot contains approximately .01 ml sample. By using a double-tiered or triple-tiered gel cover (i.e., a cover designed to hold two or three such inserts), it has been possible to separate 60 or 90 samples on a single gel of normal dimensions. In addition, it has been possible to use 40-slot inserts for the determination of haptoglobin and transferrin phenotypes. After electrophoresis, the gel is routinely sliced into 3 layers of 2 mm thickness each; recent experiments have enabled 4 slices of 1.5 mm thickness to be obtained.

Identification of Gc, Haptoglobin, and Transferrin: Prior to electrophoresis, samples were prepared in the following proportions: 0.1 ml serum: 0.005 ml hemolysate (15% hemoglobin): 0.010 ml Fe⁵⁹ Citrate solution (0.010 mg Fe/ml, 100 µc/ml). After electrophoresis the gel was sliced into three layers. The bottom layer was stained for protein with Amido black 10B; Gc phenotypes were determined from the bottom surface of this layer (i.e., from the gel surface adjacent to the lucite mold). Transferrin was determined by exposing the middle layer of the gel against Kodak No-Screen Medical X-ray film for 12 hours; to reduce diffusion of the protein bands, the gel was frozen at -10° C during the exposure. Haptoglobin was determined from the top layer of the gel by the benzidine reagent (0.2 Gm benzidine, 0.5 ml glacial acetic acid, 0.2 ml H₂O₂ (30%)) in 100 ml 50% ethanol.

Immunoelectrophoresis was carried out in agar gel according to the microtechnique of Scheidegger as modified by Hirschfeld (Scheidegger, 1955; Hirschfeld, 1959a).

Serum for identification of phenotypes in the Gc system was examined after various periods of storage at -10° C, and no difference was found from the pattern observed in freshly drawn serum. Caucasian and Negro sera were obtained as part of a Gc gene frequency survey. The Chippewa Indian samples are described in the adjoining communication (Cleve *et al.*, 1963).

RESULTS

Identification of Group-specific Components (Gc)

When starch gel electrophoresis was carried out under the described conditions, individual human sera could be classified into three common types according to their protein pattern in the region migrating immediately behind

the broad albumin band. These post-albumin patterns consisted either of a single relatively faster-migrating component, or a single relatively slower-migrating component, or a mixture of the fast and slow components. When these variations were compared with Gc phenotypes established previously by immunoelectrophoresis, it was found that the type with the faster-migrating component in the starch gel corresponded to the phenotype Gc 1-1, the type with the slower-migrating component to Gc 2-2, and the type with both components to Gc 2-1 (Fig. 1). In addition, the identity of these two post-albumin components with Gc 1 and Gc 2 has been demonstrated by comparing the electrophoretic mobilities of purified preparations of Gc 1-1 and Gc 2-2 (Cleve and Bearn, 1962) with standard sera of known Gc phenotype; the purified Gc 1-1 and Gc 2-2 preparations migrated as single components in the starch gel with mobilities corresponding to those of Gc 1 and Gc 2, as indicated in Fig. 1. Over 450 determinations of Gc phenotypes have been carried out on more than 100 different sera representing the three common phenotypes, and in each case there has been complete correspondence between the starch gel and the immunoelectrophoretic phenotypes.

It was found that the serum Gc phenotypes could be determined only from the bottom layer of the gel, and, more particularly, only from the bottom surface of the bottom layer (the starch-lucite interface). The staining pattern with Amido black through the entire thickness of the gel is illustrated diagram-

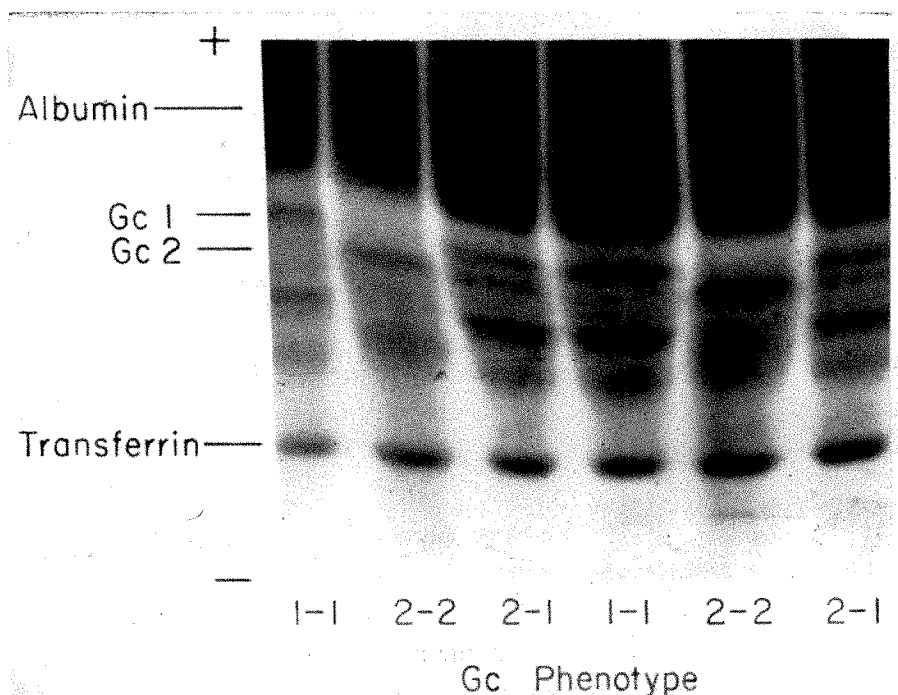


FIG. 1. Common phenotypes of the Gc system by vertical starch gel electrophoresis. Amido-black stain of bottom surface of gel. In this and subsequent photographs of starch gel patterns, only the portion of gel between transferrin and albumin is shown.

natically in Fig. 2. The properties of the starch-lucite interface are such that at this boundary the trailing (cathodal) border of the broad albumin band shows an enhanced mobility relative to the other protein components; the magnitude of this effect is an increase of approximately 20 per cent in the electrophoretic mobility of the cathodal albumin border at the bottom interface. At the top surface of the gel (the starch-cellophane-air interface), there is considerable nonspecific trailing of all protein components. The profile of the

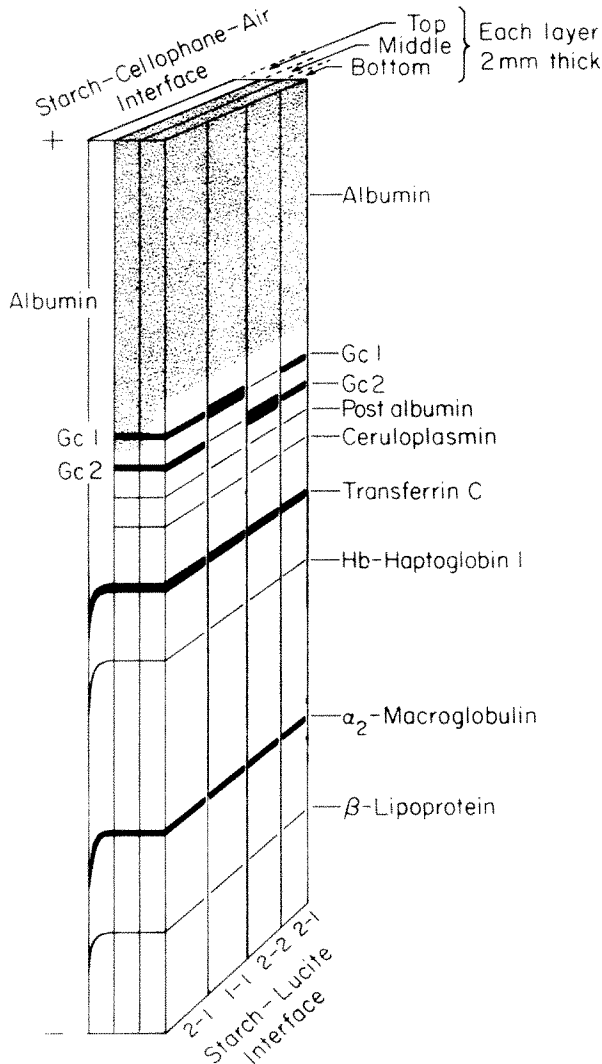


FIG. 2. Determination of phenotypes in the Gc system by vertical starch gel electrophoresis. Diagram of relative mobilities of human serum proteins in cross-section of gel and at bottom surface (starch-lucite interface). All proteins show significant trailing at the top surface (starch-cellophane-air interface), but only albumin shows the enhanced mobility effect at the bottom surface. Other hemoglobin (Hb)-haptoglobin complexes in addition to Hb-haptoglobin 1 are not shown.

trailing edge of the albumin in the cross-section of the gel therefore shows a characteristic "S-like" pattern which is not observed for any other serum protein. The specific increase of the mobility of albumin at the starch-lucite interface is sufficient to pull the trailing border of the albumin region ahead of the Gc components, so that the Gc 1 protein band can be clearly differentiated, whereas in the interior of the gel the Gc 1 band migrates within the albumin region and cannot be distinguished.

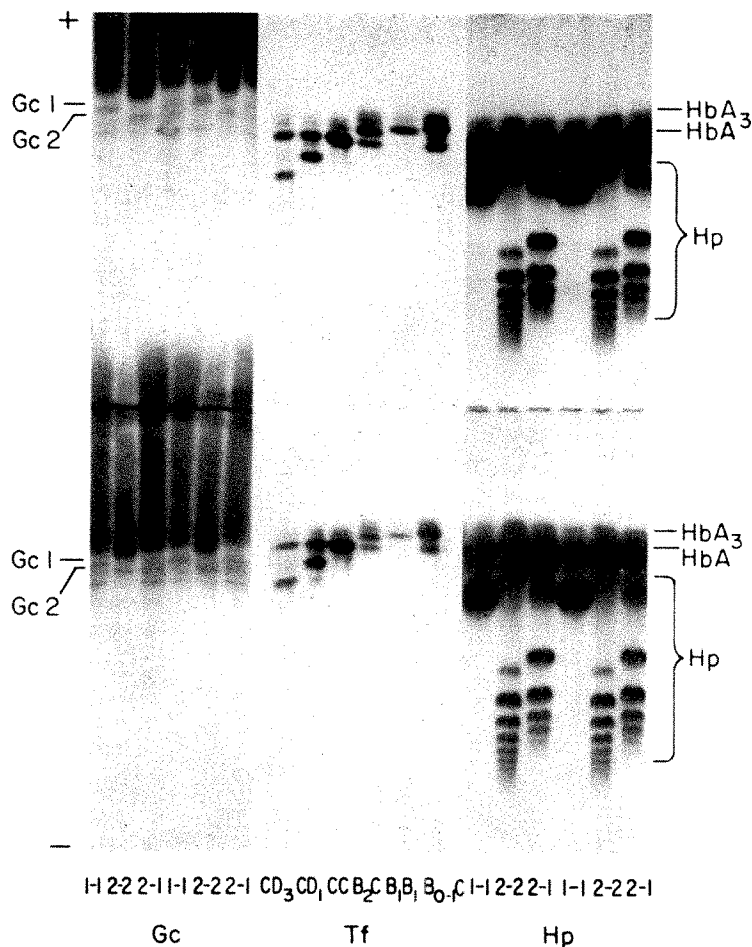


FIG. 3. Population screening of serum proteins by starch gel electrophoresis. Gels prepared in conventional vertical electrophoresis mold with cover containing two sample-insert tiers of 16 slots each. Thus, 32 samples were analyzed on these gels. After electrophoresis gels were sliced into three layers:

(left) Amido-black stain of bottom surface of gel, indicating Gc phenotypes.

(center) Autoradiograph from middle layer of gel, indicating transferrin (Tf) phenotypes. Fe^{59} added to samples prior to electrophoresis.

(right) Benzidine stain of top layer of gel, indicating haptoglobin (Hp) phenotypes. Hemoglobin added to samples prior to electrophoresis. In addition to the hemoglobin-haptoglobin complexes, excess normal adult hemoglobin (HbA_1) and a hemoglobin decomposition product (HbA_2) are also observed.

Measurement of the potential gradient along the gel at one cm intervals during electrophoresis revealed a relatively sharp peak of potential (25 volts/cm) which migrated at the trailing border of the albumin; all other portions of the gel showed approximately the same potential (20 volts/cm) throughout the run. Smithies (1955) has described similar variations in potential at lower voltage gradients (5-6 volts/cm).

Simultaneous Large-Scale Determination of Gc, Transferrin and Haptoglobin Phenotypes

Application of the high voltage technique to the screening of large numbers of sera is indicated in Fig. 3. In this experiment a double-tiered gel cover, with 16 sample slots in each tier was used, which permitted 32 sera to be analyzed in the gel. The bottom layer of the gel was stained with Amido black for the determination of Gc phenotype, and the other two slices were used for the detection of transferrin and haptoglobin. To illustrate the resolution of a variety of Gc, Tf, and Hp phenotypes, the experiment in Fig. 3 represents a composite illustration from three different starch gel experiments in which the appropriate layer was taken for each protein component. Inserts of 30 slots have now been employed in the system, with the result that 90 samples can be analyzed in a single gel. With multiple-tier gels, the albumin region from one insert series migrates into the haptoglobin region of the adjacent anodal insert series, but this effect has not interfered with the determination of haptoglobin phenotypes.

Identification of Chippewa, Caucasian, and Negro Variants in the Gc System

By the use of the high voltage system, it has been possible to resolve three Gc variants in addition to Gc 1 and Gc 2, which are the components common to all populations (Cleve and Bearn, 1961). The existence of these variants was originally suggested by slight alterations from the normal immunoelectrophoretic patterns observed during a survey of Chippewa Indian, Caucasian, and American Negro populations. The immunoelectrophoretic patterns of these variants are illustrated in Fig. 4. In the case of the Chippewa variant, by immunoelectrophoresis the double arc of the Chippewa sample was more pronounced than in the common Gc 2-1 phenotype. Examination of the serum by starch gel electrophoresis revealed the pattern shown in Fig. 5. The Chippewa phenotype contained two prominent components in the Gc region; the electrophoretic mobility of the slower-moving component coincided with that of Gc 2, whereas the mobility of the faster-migrating component was approximately intermediate between Gc 1 and the trailing border of the albumin region. An investigation of the inheritance and gene frequency of this variant is presented in the adjoining report (Cleve, *et al*, 1963); as described there, it has not yet been possible to distinguish the Chippewa variant from Gc 1 by immunoelectrophoresis.

The immunoelectrophoretic pattern of the Caucasian variant (Fig. 4) revealed a double arc which was slightly less extended toward the Gc 2 region than in the normal Gc 2-1 phenotype; a similar variant phenotype (Gc 1-X) has been

described by Hirschfeld (1962) in a Swedish and Norwegian individual. By starch gel electrophoresis (Fig. 6) it was observed that the Caucasian phenotype consisted of a faster-moving component with the mobility of Gc 1 and a slower-migrating component with a mobility slightly greater than that of Gc 2. The Caucasian phenotype has been found in only one individual.

Finally, in the Negro phenotype, which has been observed in three unrelated individuals, the immunoelectrophoretic pattern (Fig. 4) revealed a more pronounced double arc than is present in the normal Gc 2-1 phenotype, although not as prominent as in the Chippewa phenotype; Hirschfeld (1962) has observed a similar phenotype (Gc 1-Y) in an African Negro. By starch gel electrophoresis (Fig. 7) the Negro sera revealed a slower-moving component with the mobility of Gc 2 and a faster-migrating component with a mobility slightly slower than Gc 1. The observed patterns in the Negro phenotype were found to be reproducible by both immunoelectrophoresis and starch gel electro-

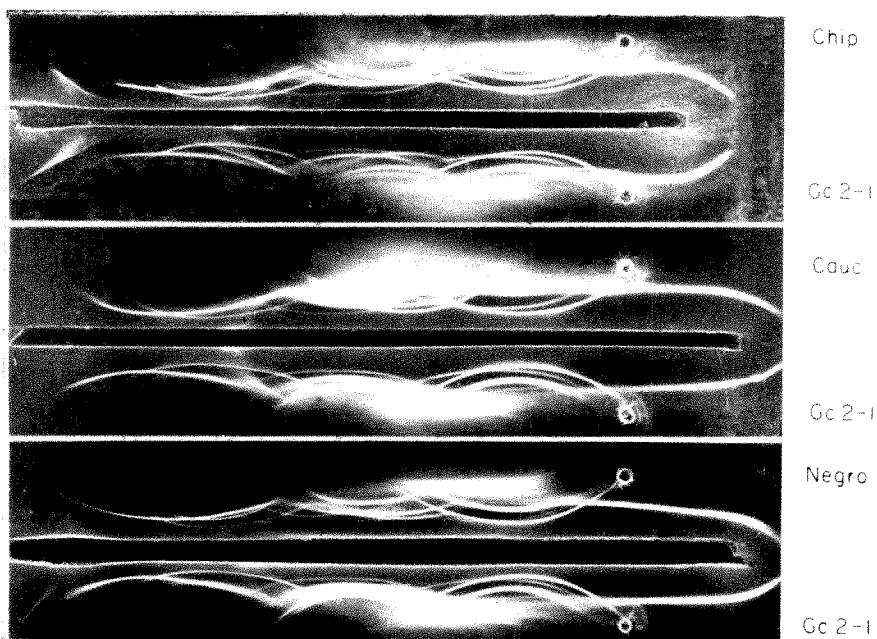


FIG. 4. Identification of variant phenotypes in the Gc system by immunoelectrophoresis of human serum. The Gc precipitin lines are the faint arcs lying closest to the antibody troughs in the middle portion of each pattern.

(top) Comparison of standard Gc 2-1 serum with a variant serum from a Chippewa Indian (Chip.). The more pronounced double arc in the variant pattern indicates the presence of a Gc protein migrating more rapidly than Gc 1 (see Fig. 5).

(center) Comparison of standard Gc 2-1 serum with a variant serum from a Caucasian individual (Cauc.). The less cathodally-extended double arc in the variant pattern indicates the presence of a Gc protein migrating more rapidly than Gc 2 (see Fig. 6).

(bottom) Comparison of standard Gc 2-1 serum with a variant serum from a Negro individual. The more pronounced double arc in the variant pattern indicates the presence of a Gc protein migrating more rapidly than Gc 1 (see Fig. 7, where, however, the Negro variant migrates more slowly than Gc 1.)

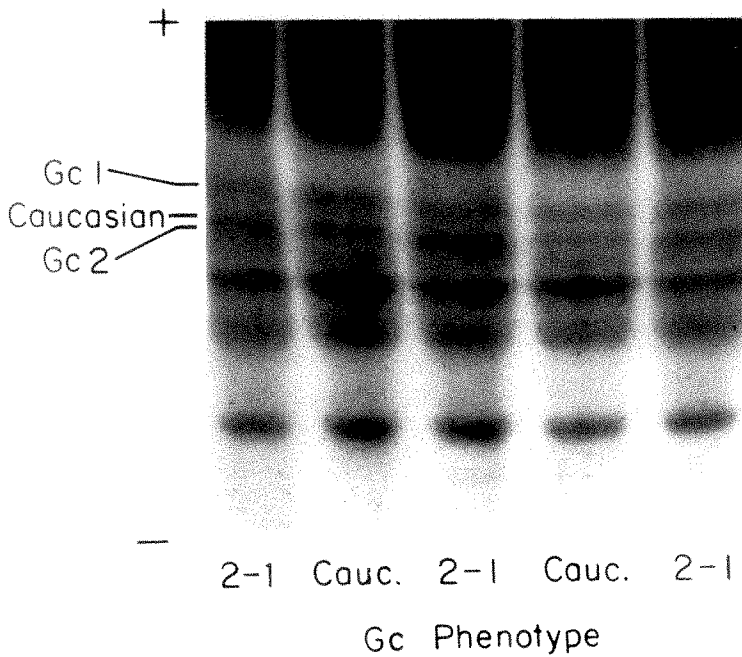
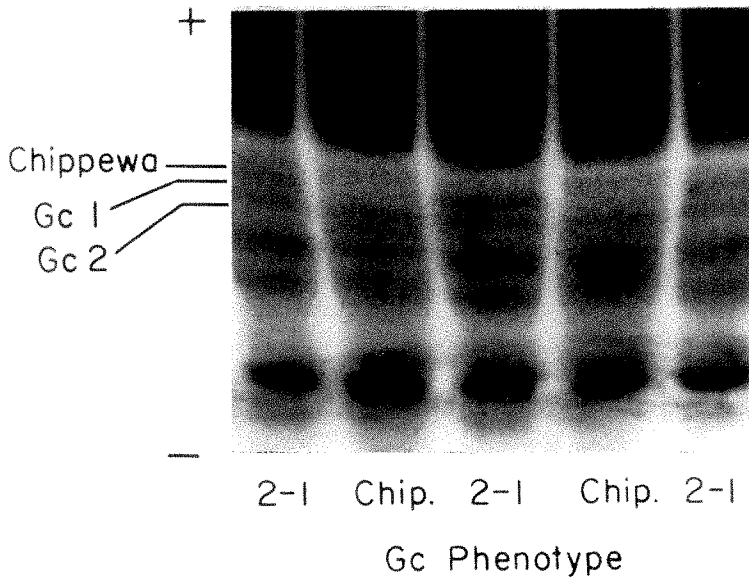


FIG. 5. Identification of Chippewa variant by starch gel electrophoresis. Comparison of standard Gc 2-1 serum with serum from a Chippewa Indian (Chip.). The Chippewa Gc band, which has a mobility intermediate between Gc 1 and albumin, appears to correspond to the more rapidly migrating component observed by immunoelectrophoresis (Fig. 4, top).

FIG. 6. Identification of Caucasian variant by starch gel electrophoresis. Comparison of standard Gc 2-1 serum with serum from a Caucasian individual (Cauc). The Caucasian Gc band, which has a mobility slightly greater than Gc 2, appears to correspond to the more rapidly migrating component observed by immunoelectrophoresis. (Fig. 4, center).

phoresis for repeat samples obtained from each of the three individuals after periods of two to four months. A similar variant phenotype was observed in serum from a brother of one of the probands. If, as appears likely, the faster migrating Negro component represents an inherited variation in the Gc system, then the mutation can be localized to an alteration in the Gc^2 rather than the Gc^1 gene, since it is in this circumstance that the relative electrophoretic mobilities by starch gel and immunoelectrophoresis are most easily reconciled. If the mutation were in the Gc^1 gene, the alteration would require the variant to migrate more rapidly than Gc 1 by immunoelectrophoresis and more slowly than Gc 1 by starch gel electrophoresis; if the mutation is in the Gc^2 gene, the alteration in mobility is in the same direction by both analytical techniques, although for reasons that are not clear the alteration is relatively greater by immunoelectrophoresis.

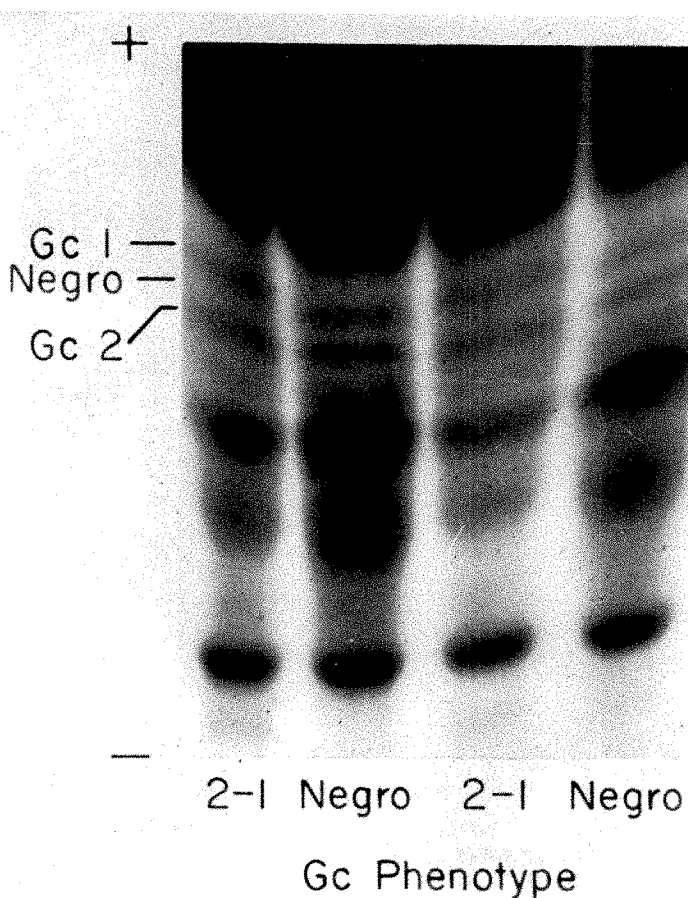


FIG. 7. Identification of Negro variant by starch gel electrophoresis. Comparison of standard Gc 2-1 serum with serum from a Negro individual. The Negro Gc band migrates slightly more slowly than Gc 1 in the starch gel pattern, although by immunoelectrophoresis it migrates more rapidly than Gc 1 (Fig. 4, bottom).

DISCUSSION

The Selective Enhancement of the Mobility of Albumin

An important aspect of the application of starch gel electrophoresis to the determination of Gc phenotypes is the unique property of the starch-lucite interface which makes this identification possible. Resolution of the Gc 1 protein band and of such faster-moving Gc proteins as the Chippewa variant is achieved only at the bottom surface of the starch gel adjacent to the lucite mold, and depends upon the selective enhancement of the mobility of albumin relative to the post-albumin components, among which are the Gc proteins. Under ordinary conditions of electrophoresis (5 volts/cm, 18 hours, 20° C), Gc 1 migrates within the albumin region. Under the high voltage conditions, (20 volts/cm, 3-4 hours, 4° C), the cathodal border of the albumin band is sufficiently increased in mobility at the lucite interface to achieve the differentiation not only of Gc 1 but also of other Gc variants, such as the Negro variant, which migrates in the Gc 1 region, and even of the Chippewa variant, which has a mobility approximately intermediate between Gc 1 and albumin. The selective advancement effect is absent in interior planes of the gel; within less than 1 mm from the bottom surface of the gel, the Gc 1 component and all faster-migrating post-albumins migrate within the albumin region. Thus, on interior planes of the gel, which are the commonly visualized surfaces after staining, Gc 2 is the component migrating closest to the albumin.

The selective advancement of albumin at the starch-lucite interface is difficult to interpret. The failure of various alternative mold surfaces to modify the observed pattern indicates that the effect is not specific for the lucite surface. It is unlikely that phenomena such as temperature dependence of mobility are responsible for this pattern. A temperature gradient exists across the thickness of the gel, since almost all of the heat transfer occurs through the air-cooled surface of the gel and not through the lucite surface; however, the temperature-dependence of albumin mobility would necessarily be remarkably different from that of other serum proteins in order to produce the observed mobility increment at the bottom surface, since no other serum protein shows a tendency for such an increased mobility. It is also unlikely that the albumin effect is caused by concentration dependence of mobility. In an experiment on the serial dilution of a serum sample the selective advancement of albumin was observed at dilutions as high as 1:64. Two tentative explanations may be offered for the albumin effect:

1. It is possible that the porous structure of the gel at the bottom surface is sufficiently increased that the molecular-sieving action of the gel is modified. A small increment in porosity could permit albumin, with a sedimentation coefficient of 4.6 S, to migrate more easily through the gel, whereas the effect may be negligible for smaller molecules such as Gc (4.1 S), which may already be moving relatively freely through the gel. The observed alterations in the relative mobilities of albumin and Gc 1 at various starch concentrations from 11 to 15 per cent may be attributed to such changes in gel porosity. However, it is unlikely that the enhanced mobility of albumin at the lucite interface results

from a change in porosity at the interface. The observation that no serum protein except albumin shows such a mobility increment requires that the effect be highly specific and operate over a narrow molecular weight range, since transferrin (MW = 83,000), haptoglobin-1 (MW = 80-100,000) and hemoglobin (MW = 68,000) fail to exhibit even a slight increase of mobility at the bottom surface. Considerable variation in the preparation of the gel failed to alter the observed pattern and suggests that the effect cannot easily be attributed to a modification of the gel structure.

2. Alternatively, the selective mobility increment of albumin may indicate that chromatography is taking place in the starch gel in addition to separation based on molecular charge and size. Since the charge structure at the lucite interface is undoubtedly different from that within the gel, it is likely that chromatographic separation would also be affected at the interface. The altered chromatographic relationship would then be specific for albumin, since the interface mobility of other serum proteins is not affected. It is possible that chromatographic retardation of albumin within the starch gel may be significantly decreased at the lucite interface, so that the mobility of the protein at the interface is increased. It is clear, however, that the precise explanation for the selective advancement of albumin at the starch-lucite interface must await further experimentation.

Relationship of the Gc Proteins to Other Post-Albumin Components in the Starch Gel Pattern

From the starch gel patterns obtained in the present study (Figs. 1, 5, 6 and 7) it is seen that between transferrin and the trailing border of the albumin there are four prominent components which can be resolved in all sera. In order of increasing mobility, these components are: ceruloplasmin, an unidentified post-albumin band, Gc 2, and Gc 1. Smithies (1959a) has demonstrated individual variations in the post-albumin region of whole serum after electrophoresis in borate buffer; comparison of these serum patterns with those of the present study indicates that the variations of Smithies are probably not related to the Gc system. The possibility that such variations may represent an additional heterogeneity in the post-albumin region is complicated by the likelihood that the faster-moving component in the variations observed by Smithies is the Gc 2 band, whereas the slower-moving component is probably unrelated to Gc.

In addition to the four principal components resolved between transferrin and albumin, a post-albumin band which migrates immediately behind Gc 2 has been observed in some sera; this component is clearly seen in the serum of the Chippewa individual (Fig. 5). A splitting of the protein band in the ceruloplasmin position into two components has also been observed, and further studies are in progress to clarify this heterogeneity.

Determination of Gc Phenotypes by the Starch Gel Technique

The use of starch gel electrophoresis for the determination of Gc phenotypes presents certain advantages over the immunoelectrophoretic method, such as the

simultaneous analysis of numerous samples and the reduction in time required for the determination. In addition, the starch gel method should facilitate the identification of Gc variants, since relative electrophoretic mobilities are more easily compared in the starch gel system (*e.g.*, the elaborate polymorphism of human transferrin: Parker and Bearn, 1962) than by immunoelectrophoresis. The similarity between the immunoelectrophoretic patterns obtained for the variant Caucasian and Negro phenotypes in the present study to those observed by Hirschfeld (1962) makes it likely that the characteristic protein components in the starch gel patterns (Figs. 6 and 7) correspond, respectively, to the X and Y components in the Hirschfeld classification of Gc variants. It is also possible that the combination of immunoelectrophoretic and starch gel techniques will increase the scope of experiments directed at the elucidation of the presently unknown biological role of the Gc system.

The identification of the Gc proteins depends partly upon the intensity of the Gc bands relative to the intensities of the other post-albumin components. In sera of phenotype Gc 2-2, a faint component is present which migrates in the Gc 1 position; correspondingly, in sera of Gc 1-1 phenotype, a faint component is present which migrates in the Gc 2 position. In the following communication (Cleve, *et al.*, 1963) a weak post-albumin component, apparently unrelated to the Gc system, is described which migrates in the position of the Chippewa variant. These minor components have not been identified, although certain α_1 - and α_2 -globulins are known to migrate in the post-albumin region of the starch gel (Cleve and Bearn, 1962). Possible confusion in Gc typing of serum may occur if one of the faint components is selectively increased; for example, a Gc 2-2 serum could be interpreted as Gc 2-1. However, the large number of sera which have been compared by immunoelectrophoresis and starch gel electrophoresis suggests that this possibility is unlikely. A similar difficulty could arise in the identification of a hypothetical Gc variant migrating more slowly than Gc 2, since in such a case, the Gc band could be obscured by the slower-moving post-albumin component which is not part of the Gc system, but which has an intensity approximately equal to that of the Gc proteins. However, in the large number of sera which have now been examined by immunoelectrophoresis in many laboratories, no example of a variant migrating more slowly than Gc 2 has been found. A further potential difficulty in identification of Gc proteins by starch gel electrophoresis is the possibility that certain variants may migrate within the albumin region. In the adjoining report (Cleve *et al.*, 1963) a variant is described in an Aborigine population which by immunoelectrophoresis appears to migrate slightly more rapidly than the Chippewa variant. The starch gel patterns of individuals homozygous by immunoelectrophoresis for the variant reveal no definite Gc band. Punch experiments described in that report indicate the presence of a Gc component within the trailing portion of the albumin region. It therefore appears that the most reliable method for determination of Gc phenotypes is by a conjunction of the starch gel and immunoelectrophoretic techniques. Under present conditions, neither method alone is capable of detecting all of the known phenotypes.

The increased capacity of the gel for rapid determination of multiple serum

phenotypes is of interest in human genetics when relatively large numbers of individuals are available for population studies at the biochemical level. Results of the present study indicate that it is feasible for one person using three gels to screen 270 samples of human serum per day by starch gel electrophoresis. The practicability of slicing each gel into four layers permits simultaneous analysis of the samples by diverse enzyme and protein stains.

SUMMARY

A method is described for the determination of Gc (group-specific component) phenotypes by starch gel electrophoresis of human serum. By electrophoresis under conditions of high voltage and low temperature, the Gc 1-1, Gc 2-1, and Gc 2-2 phenotypes are readily identified. The method has also been applied to the identification of variants in the Gc system in addition to Gc 1 and Gc 2; thus, characteristic post-albumin components which correspond to variant Gc phenotypes observed by immunoelectrophoresis have been found in Chippewa Indians and in Caucasian and Negro populations.

Detection of Gc bands with a mobility equal to or greater than that of Gc 1 is dependent upon a specific enhancement in the mobility of albumin at the interface between the gel and the plastic supporting mold; the effect is consistently observed under high voltage conditions in borate buffer, and enables rapid and reproducible identification of Gc types to be made.

A method is also described which permits the convenient analysis of large numbers of samples in a single gel, which can be sliced into multiple layers for utilization of specific staining techniques.

Note added in proof. Sept. 7, 1963.

Since this paper was completed Nerstrøm and Skafte-Jensen (1963) have reported that under certain unfavorable storage conditions, the normal Gc pattern may be modified to produce a more rapidly migrating arc in immunoelectrophoresis. As discussed in the adjoining report (Cleve *et al.*, 1963), it is extremely unlikely that such a transformation is responsible for the Chippewa variant. It is also unlikely that such an effect could account for the Negro variant, since the Negro phenotype could be clearly identified by starch gel electrophoresis. Those sera in the present experiments in which immunoelectrophoretic alterations similar to those of Nerstrøm and Skafte-Jensen could be induced by unfavorable storage conditions revealed no Gc component corresponding to the Negro variant in the starch gel system.

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Two Genetic Variants of the Group-Specific Component of Human Serum: Gc Chippewa and Gc Aborigine

H. CLEVE, R. L. KIRK, W. C. PARKER, A. G. BEARN,
L. E. SCHACHT, H. KLEINMAN, AND W. R. HORSFALL

*The Rockefeller Institute, New York, New York, State of Minnesota Department of Health,
Minneapolis, Minnesota and Commonwealth Department of Health, Cairns, Australia*

THE GROUP-SPECIFIC COMPONENTS (Gc) are serum α_2 -globulins which comprise a human serum protein polymorphism distinguishable by immunoelectrophoresis (Hirschfeld, 1959). Variations in relative electrophoretic mobility permit the classification of individual sera into three common phenotypes. Some sera contain a fast migrating component (Gc 1-1), others a slow migrating component (Gc 2-2), while the third phenotype is characterized by the presence of both components in approximately equal amounts (Gc 2-1). The common phenotypes are controlled by two autosomal co-dominant alleles, Gc^1 and Gc^2 , (Hirschfeld, Jonsson and Rasmuson, 1960; Cleve and Bearn, 1961; Reinskou and Mohr, 1962).

In the course of studies on the distribution of the Gc alleles in various populations, a number of unusual Gc phenotypes have been observed (Hirschfeld, 1962a, b; Cleve and Bearn, 1962). One phenotypic variation has occurred among Caucasians and consists of a variant which migrates slightly more rapidly than Gc 2-2 and thus lies intermediate between Gc 1-1 and Gc 2-2. This variant has been termed Gc X (Hirschfeld, 1962a). Another variation has been found in the serum of an African Negro. In this instance, the variant migrates slightly more rapidly than Gc 1-1, and has been called Gc Y (Hirschfeld, 1962a). Similar and possibly corresponding variants have been observed in our laboratory in one Caucasian subject and three American Negroes. Further identification of these variants using the additional technique of starch gel electrophoresis has been described in the preceding communication (Parker, Cleve and Bearn, 1963). It has not yet been possible to collect sufficient family material to test the hypothesis that these two rare phenotypic variations are under genetic control, and further investigations are in progress.

In the present report, two new genetically determined variants of the Gc-system will be described. The first was observed in a sample of Chippewa Indians during a study of Gc gene frequencies in North American Indians. The second variant was disclosed in a sample collected in the Cape York area of northeastern Australia during the course of an extensive survey of the Australian Aborigine population. These two variants are designated, therefore, as Gc

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Chippewa and Gc Aborigine, respectively. Both variants were originally detected by immunoelectrophoresis, but the recognition of certain phenotypes involving the variant Gc Chippewa was made possible only by application of the modified starch gel procedure. This investigation includes the results of family studies and the determination of the gene frequency of the two alleles in the populations where they were first observed.

METHODS

Immunoelectrophoretic analysis: Sera were examined according to Scheidegger's micro-method as modified by Hirschfeld (Scheidegger, 1955; Hirschfeld, 1959a). A voltage gradient of 6-7 volts/cm was applied for 120 minutes. The antisera used in this study included horse antisera No. 13411 and No. 306 from the Institut Pasteur, Paris, and several rabbit antisera prepared in our laboratory.

The validation of suspected variants of the Gc types by immunoelectrophoresis requires the examination of mixtures of the proband serum with standard sera of known Gc type. The mixtures were obtained by inserting into the origin 1 μ l of the proband's serum followed by 1 μ l of the standard serum.

Starch gel electrophoresis: The vertical starch gel system of Smithies was utilized with modifications to improve resolution of the Gc bands, as described by Parker, Cleve and Bearn (1963).

CHARACTERIZATION OF THE NEW GENETIC VARIANT Gc CHIPPEWA

Material

The Chippewa Indians are members of the Algonquian linguistic group of North American Indians. Originally, they lived as nomadic hunters and fishers in the Great Lakes region on the northern shores of Lake Huron and in the area surrounding Lake Superior. In the United States today they live on several reservations scattered throughout the northern part of Minnesota, where many are still engaged in their traditional pursuits of fishing and hunting.

The specimens were collected on the Red Lake Reservation, Red Lake, Minnesota. Blood was drawn under sterile conditions into evacuated glass containers (Vacutainer) and sent to New York City. The material arrived in the laboratory within 3 days. In the original survey, 159 individuals were sampled, including some related persons and persons with varying degrees of non-Indian ancestry. For gene frequency analysis, all individuals with recorded non-Indian ancestry were excluded; the remaining sample of full blood Chippewa Indians consisted of 62 individuals. From four individuals of the original sample in whom an unusual Gc phenotype was observed, it was possible to secure a second blood specimen. These four individuals were members of a single kindred. Specimens from 36 additional members of this kindred were obtained and more detailed genealogical information was obtained.

Results

One hundred fifty-nine sera of Chippewa Indians were examined by immunoelectrophoresis and the distribution of the Gc phenotypes was determined. Ninety-four sera were provisionally classified as Gc 1-1, fifty-nine sera as Gc 2-1, and six sera as Gc 2-2. The frequency of Gc² was calculated to be 0.223.

It was noticed, however, that several sera provisionally classified as Gc 1-1 and Gc 2-1 revealed an unusual immunoelectrophoretic pattern. In five of 59 sera classified as Gc 2-1, the pattern was clearly distinguishable from the usual heterozygous pattern. As illustrated in Fig. 1, the Gc precipitate in these sera showed a more distinct separation between the peaks of the fast and slow-migrating components than in the usual heterozygous Gc type. The fast component in such sera migrated slightly more rapidly than Gc 1, thereby causing an extension of the precipitate towards the anode. This phenotypic variation was technically reproducible. In some sera, provisionally classified as Gc 1-1, it was noticed that the Gc precipitate seemed to be slightly more extended towards the anode than commonly observed in Gc 1-1 sera, (Fig. 1), but the deviation was small and a clear cut distinction from the common Gc 1-1 type was not obtained.

A modification of the vertical starch gel electrophoresis technique of Smithies (Parker, Cleve and Bearn, 1963) has provided an additional method for the determination of Gc types in human sera. The group-specific components migrate on starch gel in the post-albumin region. The product of the Gc^1 allele is present in the first prominent post-albumin band; the product of the Gc^2 allele is present in the second. Additional post-albumin bands are not related to the Gc-system. Application of this technique to the investigation of the variant types observed in Chippewa Indians permitted a further characterization. The starch gel electrophoretic pattern of the variant types is illustrated in Fig. 2. A prominent band located between the cathodal edge of the albumin band and the Gc^1 band was a characteristic finding in each variant. In the sera originally classified as unusual Gc 1-1 types this distinct band was present together with a prominent band in the Gc^1 position; in the sera originally classified as unusual Gc 2-1 types the band in the fast migrating position appeared together with a prom-

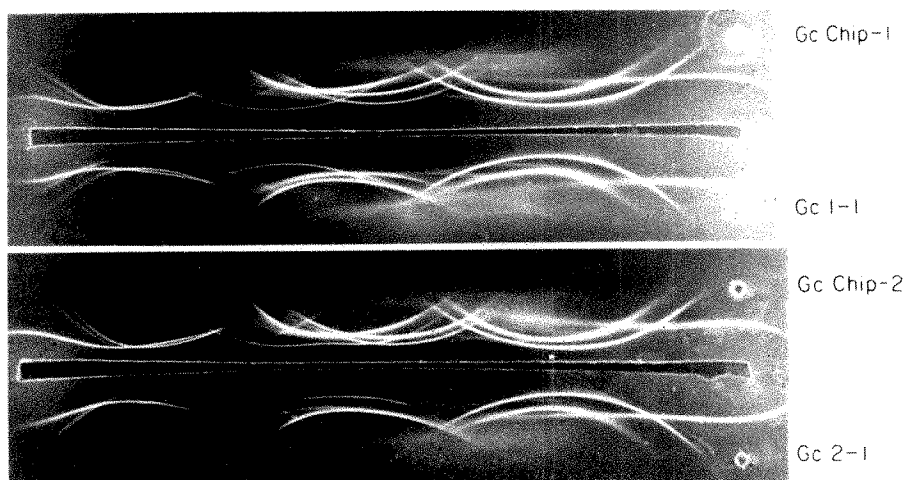


FIG. 1. Immunoelectrophoretic analysis of sera from Chippewa Indians. The phenotype Gc Chippewa-1 is compared with the standard Gc 1-1, and the phenotype Gc Chippewa-2 is compared with the standard Gc 2-1. Note the differences in shape and position of the Gc precipitates between the usual and the variant phenotypes.

inent band in the Gc² position. The phenotypic appearance of the variant types on immuno- and starch gel electrophoresis indicates the presence of a faster migrating variant of the group-specific component, which is designated Gc Chippewa. The two unusual Gc types were classified Gc Chippewa-1 and Gc Chippewa-2 (Gc Chip-1; Gc Chip-2). In sera of the common Gc types, a weak post-albumin component can be seen in a position corresponding to the prominent Gc Chippewa band. From four individuals typed as Gc Chippewa-2, a second sample of blood was obtained after an interval of three months; the phenotypic variation was found to be reproducible. Because of the difficulty in distinguishing between Gc Chip-1 and Gc 1-1 by immunoelectrophoresis, all sera provisionally classified as Gc 1-1 by immunoelectrophoresis were re-examined by starch gel electrophoresis. Of 38 such sera, 10 were classified as Gc Chip-1 and 28 as Gc 1-1.

The genetic control of the Chippewa variant was tested by determination of Gc types in the sera of 41 members of three generations of a large Chippewa kindred (Fig. 3). The kindred contains several informative matings. In four families both parents and a total of 14 offspring were investigated; in four other families, with a total of 14 offspring examined, only one parent was available in each family. In addition, the kindred contains two mother-child combinations. The variant Gc Chippewa was inherited in a pattern consistent with an autosomal, co-dominant allele *GcChippewa* (*GcChip*) at the Gc locus; no exceptions to simple mendelian inheritance were found.

The distribution of the Gc alleles was studied in the sample of 62 fullblood

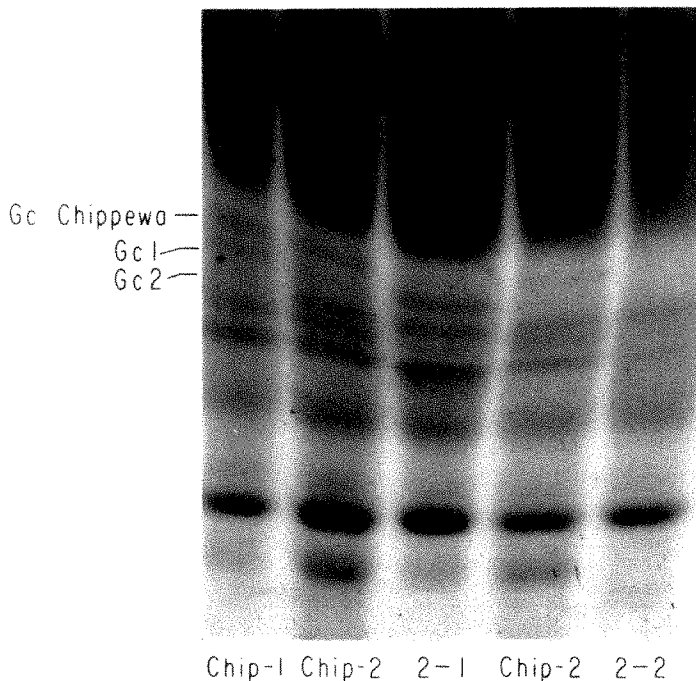


FIG. 2. Demonstration of Gc Chippewa phenotypes by starch gel electrophoresis.

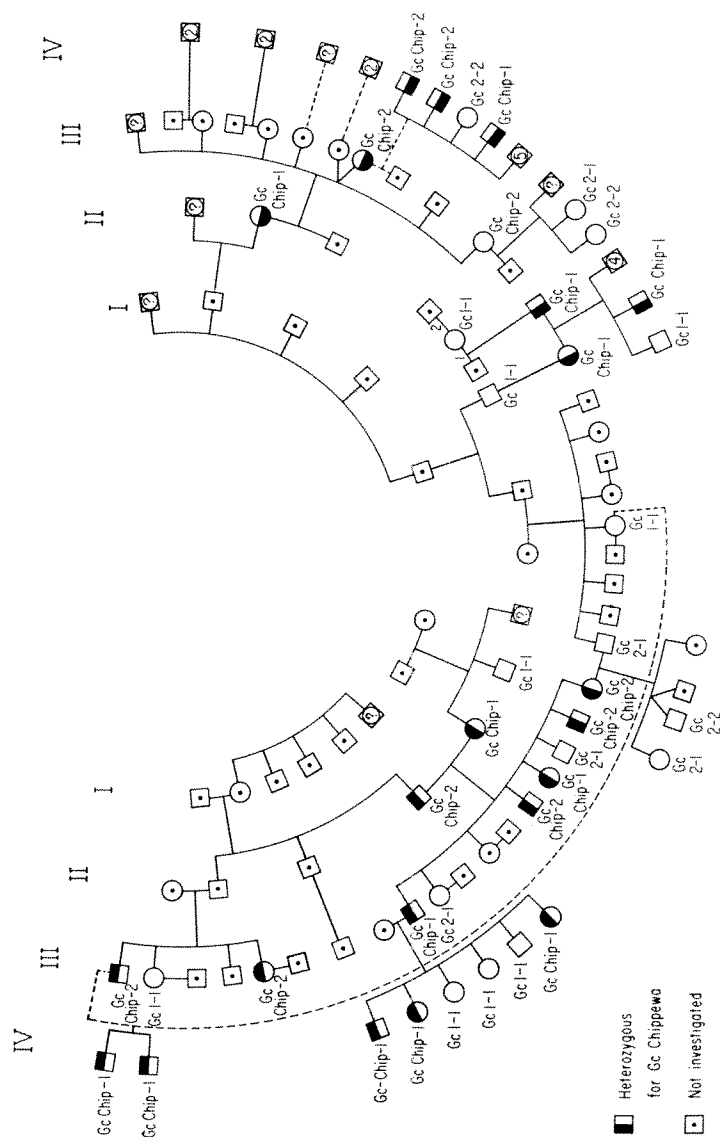


FIG. 3. Pedigree of a kindred of Chippewa Indians illustrating the inheritance of Gc Chippewa.

TABLE 1. DISTRIBUTION OF Gc ALLELES IN 62 CHIPPEWA INDIANS

	Total	Gc Chip-Chip	Gc Chip-1	Gc Chip-2	Gc 1-1	Gc 2-1	Gc 2-2
Obs.	62	0	10	3	28	19	2
Exp.	61.99	0.68	8.92	2.73	20.09	17.84	2.73

Gene Frequencies:

$Gc^{Chip} = 0.105$

$Gc^1 = 0.685$

$Gc^2 = 0.210$

$\chi^2 \text{ corrected (Yates)} = 0.1605$

$P > .50$

Chippewa Indians; the results are summarized in table 1. The frequencies of the common alleles were calculated to be 0.685 for Gc^1 and 0.210 for Gc^2 . The frequency of the variant allele $Gc^{Chippewa}$ was 0.105. The observed distribution of phenotypes in this small sample was in good agreement with the expected distribution on the basis of the Hardy-Weinberg equilibrium.

CHARACTERIZATION OF THE GENETIC VARIANT Gc ABORIGINE

Material

During an extensive survey of the distribution of Gc alleles in Australian Aborigines, unusual Gc phenotypes were observed in samples collected at the Aurukun Presbyterian Mission. The Aurukun Mission is situated near the mouth of the Archer River on the west coast of the Cape York Peninsula. The Mission is in contact with several hundred Aborigines. The majority of these individuals belong to a group of tribes whose names begin with Wik, particularly the Wik-Mungken, Wik-Eppa, Wik-Ngartona and Wik-Ngencherra. Inter-marriage between these groups has been frequent in recent years and at present the entire group is considered as a single unit.

Blood of 74 full blood Aborigines was collected, sent by air to Perth, Western Australia, and the serum stored at -20°C . Aliquots of these samples were sent in dry ice to New York City. It was possible to obtain a second sample from two of the individuals who showed unusual phenotypic variations.

Results

Sera from the 74 individuals were examined by immunoelectrophoresis. Careful analysis of the Gc precipitates in these sera revealed six different Gc phenotypes (Fig. 4) of which three were previously unrecognized. The three unusual Gc phenotypes differ from the common types in possessing a more rapidly migrating variant of the group-specific component called Gc Aborigine. It became apparent that this variant is controlled by an additional allele at the Gc locus, $Gc^{Aborigine}$ ($GcAb$). The homozygous type Gc Aborigine-Aborigine ($Gc\ Ab\text{-}Ab$) contains a group-specific component migrating distinctly faster than $Gc\ 1\text{-}1$. The precipitate is located in the α_1 -globulin region and is arc-shaped in a fashion similar to the precipitates of the homozygous types $Gc\ 1\text{-}1$ and $2\text{-}2$. The sera of the heterozygous type Gc Aborigine-1 ($Gc\ Ab\text{-}1$) show a flatter, extended precipitate covering the area of the faster migrating component and $Gc\ 1\text{-}1$. The sera of the type Gc Aborigine-2 ($Gc\ Ab\text{-}2$) contain a precipitate representing the faster migrating component and the gene product of Gc^2 . The precipitate is extended from the α_1 -globulins to the slow α_2 -globulins and reveals two distinctly separated peaks. The position of the anodal peak corresponds to the maximum of the faster migrating component, Gc Aborigine; the position of the cathodal peak corresponds to the location of $Gc\ 2\text{-}2$. The phenotypic variations were technically reproducible and were verified by comparative analysis with sera of standard Gc types. Additional confirmation was obtained by the investigation of mixtures of sera with variant types and

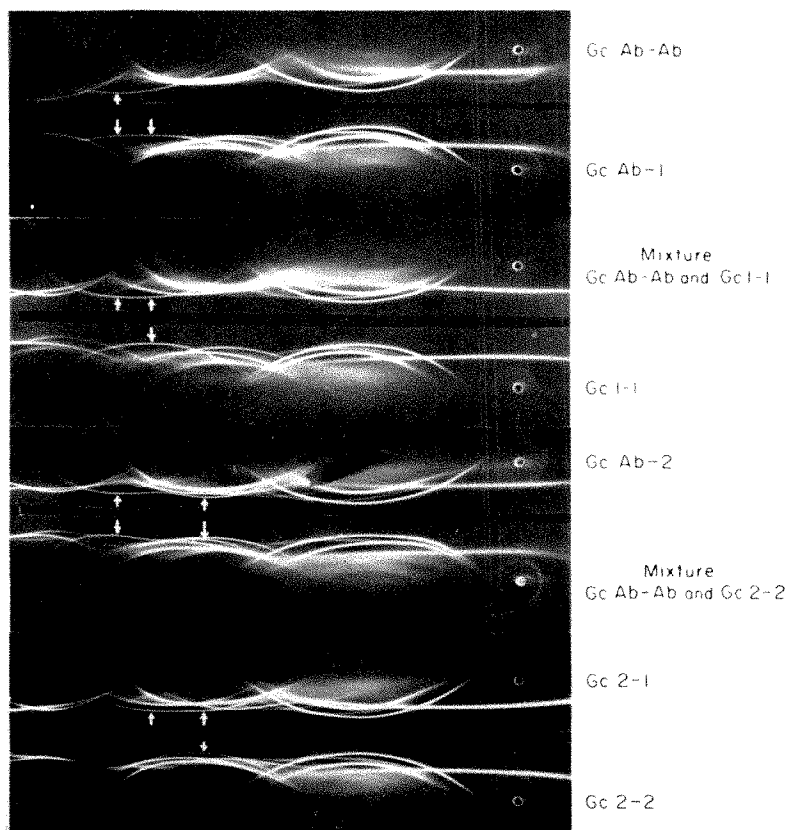


FIG. 4. Immunoelectrophoretic demonstration of the genetic variant Gc Aborigine. The figure shows the common Gc types 1-1, 2-1 and 2-2 and the variant phenotypes Ab-Ab, Ab-1 and Ab-2. The results of mixing the homozygous variant type with homozygous standard sera are also illustrated.

sera with common Gc types. Sera with suspected variant phenotypes were classified only after repeated examination by comparative analysis and by investigation of artificial mixtures. Mixtures of Gc Ab-Ab serum with standard sera of type Gc 1-1 and type Gc 2-2 are also illustrated in Fig. 4. The former reveals a Gc phenotype equivalent to the heterozygous type Gc Ab-1; the latter has the phenotype of the heterozygous type Gc Ab-2.

Comparative immunoelectrophoretic analysis of the variants Gc Chippewa and Gc Aborigine revealed minor differences in relative mobilities. By prolonged electrophoresis (120-140 minutes) Gc Aborigine was observed to migrate slightly more rapidly than Gc Chippewa. By starch gel electrophoresis, a clear difference between the Chippewa and Aborigine variants was disclosed. As previously described, Gc Chippewa migrates between Gc 1 and albumin. A protein band corresponding to Gc Aborigine could not be detected by inspection of the protein-stained starch gel. For the localization of Gc Aborigine, a series of punches (diameter 0.35 cm) extending from the region of the albumin to ceruloplasmin was taken from the bottom slice of the unstained gel. The punches

were re-examined by immunoelectrophoresis on agar gel and Gc Aborigine was localized in the punches taken from the cathodal edge of the albumin band. It may therefore be concluded that using the electrophoretic conditions employed in this experiment, Gc Aborigine migrates within the albumin region.

To test the proposed genetic hypothesis, only a single informative family was available (Fig. 5). Both parents were heterozygous for Gc Aborigine. Among the eight children of this mating all of the possible Gc phenotypes were observed (Gc Ab-Ab, Gc Ab-1, Gc Ab-2, Gc 2-1). It was possible to secure a second sample of serum from the father (Gc Ab-1) and a homozygous daughter (Gc Ab-Ab) after an interval of 15 months. In both sera the demonstration of the phenotypic variation was reproducible and the original classification was confirmed.

The distribution of Gc phenotypes in the sample of 74 aborigines from the

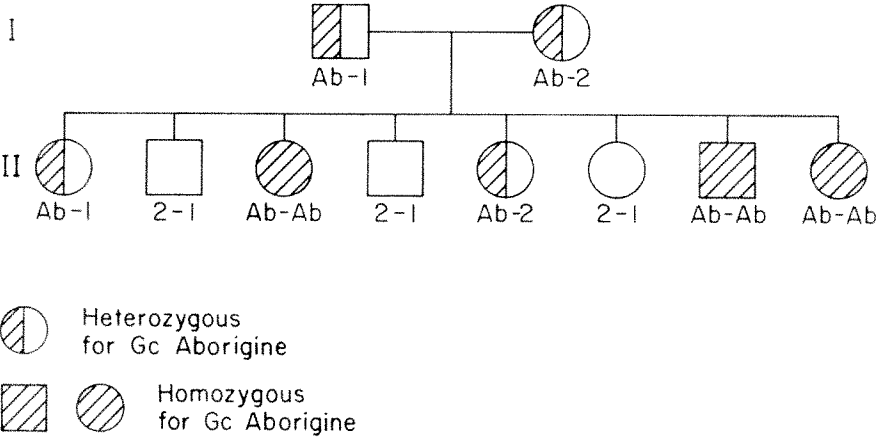


FIG. 5. Inheritance of Gc Aborigine in a kindred from the Cape York area (Aurukun).

TABLE 2. DISTRIBUTION OF GC ALLELES IN A GROUP OF AUSTRALIAN ABORIGINES FROM AURUKUN

	Total	Gc Ab-Ab	Gc Ab-1	Gc Ab-2	Gc 1-1	Gc 2-1	Gc 2-2
Obs.	74*	1	7	1	42	18	5
Exp.	74	0.34	7.41	1.97	40.09	21.35	2.84

Gene Frequencies:
Gc^{Ab} = 0.068
Gc¹ = 0.736
Gc² = 0.196
 χ^2 corrected (Yates) = 1.5888
P > .20
*Includes family material.

	Total	Gc Ab-Ab	Gc Ab-1	Gc Ab-2	Gc 1-1	Gc 2-1	Gc 2-2
Obs.	54*	0	4	1	32	13	4
Exp.	53.99	0.11	3.73	1.01	30.37	16.53	2.25

Gene Frequencies:
Gc^{Ab} = 0.046
Gc¹ = 0.750
Gc² = 0.204
 χ^2 corrected (Yates) = 1.6811
P > .10
*Corrected for family material.

Aurukun Mission is shown in the upper part of table 2. Since this sample contained family material, a correction was made by excluding all children when a parent was examined, and all sibs except the first sib when more than one was examined. The distribution of Gc types in the remaining sample of 54 individuals is given in the lower part of table 2. The Gc frequencies were found to be: $Gc_{Aborigine} = 0.046$; $Gc^1 = 0.750$ and $Gc^2 = 0.204$. The observed and expected distributions, assuming equilibrium, were in close agreement.

DISCUSSION

During the past 3 years information has accumulated rapidly on the distribution of the alleles Gc^1 and Gc^2 in populations from different parts of the world. The observed differences have been reviewed (Cleve and Bearn, 1962). It was found that Gc^1 was more common than Gc^2 in all populations studied. The highest Gc^2 frequencies were 0.34, observed in a sample of 99 Ashkenazic Jews from Israel, and 0.31, found in a sample of 90 Asiatic Indians from Bombay. The lowest frequency of the Gc^2 allele, 0.02, was reported in a Navajo Indian sample of 245 individuals. European populations range from 0.29 in England to 0.23 in Denmark, while the frequency of Gc^2 is significantly lower in African Negroes, where values between 0.10 and 0.05 have been observed. In samples from Chinese and Japanese populations, the frequencies were similar to those in Europeans (0.23 in both populations).

The recognition of variations of the common Gc types in certain Caucasians and African Negroes by Hirschfeld and ourselves suggested the possible existence of additional rare alleles at the Gc locus, (Hirschfeld, 1962a and b; Parker, Cleve and Bearn, 1963). The disclosure of two new inherited variations in Chippewa Indians and Australian Aborigines constitutes evidence for a more complex genetic polymorphism of the Gc-system. Both variants are distinguishable from the common group-specific components by their differences in relative electrophoretic mobilities, and both represent variants which are faster migrating than the product of the Gc^1 allele. Results of the family studies discussed above indicate that Gc_{Chip} and Gc_{Ab} behave as alleles of Gc^1 and Gc^2 . It seems reasonable to assume that both variants originated by structural mutations of one of the common alleles, more likely by a mutation of Gc^1 than of Gc^2 . Although the isolation and partial characterization of the gene products of the alleles Gc^1 and Gc^2 has been achieved recently (Cleve and Bearn, 1962), the methods available at present do not permit their preparation in quantities sufficiently large to determine the precise nature of the structural differences between the group-specific components. It seems probable that the observed variations in electrophoretic mobilities are due to differences in charge rather than to differences in size of the protein molecules.

The characterization of variant types in the Gc-system has been facilitated by application of a modified starch gel electrophoretic technique (Parker, Cleve, and Bearn, 1963). The high resolving power of this procedure permits the recognition of minute differences in relative electrophoretic mobilities which are difficult to disclose by immunoelectrophoresis on agar gel. For example, the starch gel technique has permitted the identification of the variant Gc Chippewa

in the presence of Gc 1 (Gc Chip-1), which was not possible by immunoelectrophoresis. In the screening of large samples for the determination of Gc types in human populations, starch gel electrophoresis appears to be a valuable method. It should be pointed out, however, that Gc Aborigine and other faster migrating variants with a mobility similar to albumin could be overlooked if the investigation is based solely on examination by the present methods of starch gel electrophoresis.

Variations of the Gc-system appear to be characteristic of certain populations or of particular geographic areas. Gc X has been found only in Caucasians and Gc Y only in African and American Negroes. The information on the distribution of the GcChip allele is limited. No further populations of the Algonquian linguistic group have been examined and only two other distant Indian populations have been studied. GcChip was absent in a sample of 152 sera from Alabama-Coushatta Indians. These two ethnically closely related tribes, both of which belong to the Muskogean linguistic group, came originally from the Alabama River region and live today as a single unit partly in Texas, where this sample was collected, and partly in Oklahoma. The frequencies found for Gc¹ and Gc² were 0.86 and 0.14. Also, in a sample of 245 Navajo Indians no evidence for the presence of GcChip was obtained. But in this sample the frequency of Gc² was strikingly low (0.02) (Cleve and Bearn, 1961). The sample contained only nine Gc 2-1 phenotypes. Since in our experience with immunoelectrophoresis the occurrence of unusual Gc 2-1 phenotypes first draws the attention of the investigator to the possibility of the presence of Gc variants within a population, it is conceivable that faster migrating variants escaped notice in this population.

Studies on the distribution of the allele GcAb are discussed in detail elsewhere (Kirk, Cleve and Bearn, 1963). The highest values for GcAb were found in the Cape York area, with a gradual decline to the south and west to values of zero or almost zero in the region of the Western Desert. The pattern of distribution of GcAb on the Australian continent is in agreement with gene frequency variations observed for other genetic markers and suggests that the mutant has been in existence for a long period of time, before the present distribution of the Aboriginal population on the Australian continent took place. GcAb was also found in several sera in a heterogeneous sample from New Guinea, but not in a number of populations from South and South East Asia (Kirk, Cleve and Bearn, 1963). Although more extensive information is needed on the distribution of GcAb in Melanesian populations, it may prove to be a particularly useful marker for the study of genetic relationships between Australian Aborigines and populations from New Guinea.

SUMMARY

1. Two genetic variants in the Gc-system have been found. One occurred in a group of Chippewa Indians and has been named Gc Chippewa; the other was first observed in a population of Australian Aborigines and has been termed Gc Aborigine. The variants differ from the usual group-specific components in

their relative electrophoretic mobilities on agar and starch gel. Both variants migrate more rapidly than Gc 1-1.

2. Family studies indicate that the variant phenotypes behave as alleles at the Gc locus. They have been designated GcChippewa (GcChip) and GcAborigine (GcAb).

3. The frequency of GcChippewa in a sample of 62 unrelated Chippewa Indians was 0.105 and the frequency of GcAborigine in a population of 54 unrelated Australian Aborigines was 0.046.

Note added in proof. Sept. 7, 1963.

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Nerstrøm and Skafte-Jensen (1963) have recently described a gradual transformation of the normal Gc arc in immunoelectrophoresis into a substance with the mobility of an α_1 -globulin. The transformation takes place during unfavorable storage conditions of the sample. The patterns obtained by these authors were in certain respects similar to those obtained for the phenotypes Gc Chip-2 and Gc Ab-2. In order to exclude the possibility of such a transformation as the basis of the Chippewa and Aborigine variants, further samples were obtained from 21 selected individuals in the Chippewa population and from five members of the Aborigine pedigree (Fig. 5). These samples were drawn and examined under carefully regulated conditions, and in each case the previous phenotypic classification was verified. These results, together with the large amount of genetic data which has been accumulated, appears sufficient to exclude the possibility that the Chippewa and Aborigine variants can be attributed to storage or other types of non-genetic alterations in the Gc pattern.

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The ABO Blood Groups in Neoplastic Disease of the Ovary

RICHARD H. OSBORNE¹ AND FRANCES V. DE GEORGE

*Division of Preventive Medicine, Sloan-Kettering Institute for Cancer Research,
New York, New York*

STUDIES OF THE RELATIVE FREQUENCY of the ABO blood groups in various diseases have demonstrated statistical associations between this blood group system and a number of different disease entities (Clarke, 1959; Matsunaga, 1959; Roberts, 1957, 1959), but have failed to provide satisfactory explanations as to the biological basis for the associations observed (Osborne and De George, 1962, Roberts, 1959). In an earlier study, tumors of the parotid and submaxillary glands were selected for the investigation of this critical problem (Osborne and De George, 1962). It was found that blood group associations occurred with benign as well as with malignant tumors of these two glands, but only with tumors of certain histological appearances. The study of the ABO blood groups in neoplastic disease of the ovary which is reported here is a sequel of the salivary gland study.

Diseases of the ovaries were selected for the present investigation because: (a) the ovaries, like the salivary glands, are subject to a great variety of both benign and malignant neoplasms; (b) pseudomucinous cysts of the ovary contain the ABO (H) group specific substances in women who secrete these substances in their saliva; (c) carcinoma of the ovary has been reported to associate with the ABO blood group system, (Helmbold, 1961).

The purposes of the present study are to determine: (1) whether benign as well as malignant disease of the ovary associates with the ABO blood groups as in the salivary gland tumors; (2) whether the blood group frequencies differ in the various histological types of ovarian disease; (3) whether any such differences when interpreted in the light of the salivary gland experience will further elucidate the basis of blood group disease associations.

MATERIAL AND METHODS

Utilizing the 1951-1961 records of Memorial Hospital in New York City, New York, 713 cases of ovarian disease were obtained in which blood group and type of ovarian disease could be established. The number of benign and malignant cases and their classifications are given in table 1. A simple classification of ovarian neoplasms is difficult because of their varied histology and

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the frequency of transitional or mixed types (Willis, 1960). This has necessitated a hierarchical order of classification for the purposes of this study. Each case is classified by the following order of histological description: pseudomucinous, mucinous, serous, papillary, anaplastic, cystadenocarcinoma, adenocarcinoma, dermoid, teratoma, thecoma, and granulosa cell. In some instances the left and right ovaries were affected by different types of disease. For example, at surgery or autopsy a patient with papillary cystadenocarcinoma might be found to have a small dermoid cyst or focus of endometriosis on the opposite ovary. Such a case is listed as a papillary cystadenocarcinoma. In any instance where an obvious choice as to classification could not be made, the case is listed under miscellaneous. No patient is listed in more than one classification.

Blood group control values were obtained for volunteer blood donors at Memorial Hospital and are the same as used in the salivary gland study (Osborne and De George, 1962). To establish blood transfusion credit, volunteer donors are solicited by the patients and their families. Members of the patients' families constitute approximately 15 per cent of all donors, and the remainder come from their friends and work associates. Paid donors have been excluded from the control. Since all patients with ovarian disease are females, the volunteer donors are listed by sex, and as there is no difference in the blood group frequencies of male and female, the sexes are combined for the calculation of control values (table 2). In this way the arithmetic contribution of the control in the present study is the same as in the salivary gland study.

TABLE 1. CLASSIFICATION AND NUMBER OF OVARIAN NEOPLASMS

Benign		Malignant	
Classification	n	Classification	n
Pseudomucinous cyst	28	Pseudomucinous cystadenocarcinoma	17
Mucinous cyst & cystadenoma	6	Mucinous adeno-	
Simple cyst	11	& cystadenocarcinoma	15
Serous cyst	32	Cystadenocarcinoma	9
Endometrial cyst	13	Serous cystadenocarcinoma	14
Endometriosis	57	Anaplastic adenocarcinoma	20
Dermoids	47	Adenocarcinoma	103
Teratomas	9	Papillary adenocarcinoma	234
Fibroma & adenofibroma	29	Secondary carcinoma	14
Thecoma & granulosa cell	8	Granulosa cell	12
Miscellaneous*	20	Miscellaneous	15
Total	260	Total	453

*Represents different histological types with only 1-5 patients in any single classification and in those in which no classification other than benign or malignant was given.

TABLE 2. ABO BLOOD GROUP DISTRIBUTION IN THE CONTROL

			O	A	B	AB	Total
Volunteer donors	♂	n	1,728	1,561	535	201	4,025
		%	42.93	38.78	13.29	4.99	
	♀	n	301	267	101	44	713
		%	42.22	37.45	14.16	6.17	
Total		n	2,029	1,828	636	245	4,738
		%	42.82	38.58	13.42	5.17	

In the statistical analysis of the data the method proposed by Woolf (1955) is followed. By this method, the blood group associations are given as a ratio, which is the incidence of the disease in persons of one blood group relative to the incidence in persons of another blood group or groups. Chi-square with one degree of freedom is used to obtain the probability levels of these ratios.

RESULTS

In the volunteer donor control the frequency of blood group O is 42.82 per cent and that of blood group A is 38.58 per cent (table 2). In patients with ovarian disease the percentage of group O is 39.97 and that of group A is 44.04 (table 3). The frequency of ovarian disease in group A is 1.22 relative to one in group O, which is statistically significant, $P = .025$ (table 4). When benign and malignant disease are examined separately the ratios in group A are 1.29 and 1.19, respectively; neither ratio is statistically significant.

The blood group distributions in different classifications of benign and malignant neoplasms are given in tables 5 and 6. The blood group distributions differ markedly from one classification to the next. In benign disease the magnitude of the differences in the frequency of blood groups A and O are consistently greater than in comparable malignant classifications. The consistency of this pattern is reflected in the larger ratio observed for benign as compared to malignant ovarian disease, (1.29 and 1.19) (table 4).

In the benign disease classification the excess of blood group A is contributed

TABLE 3. BLOOD GROUP DISTRIBUTION IN OVARIAN NEOPLASMS

		O	A	B	AB	Total
Benign	n	98	114	35	13	260
	%	37.69	43.85	13.46	5.00	
Malignant	n	187	200	47	19	453
	%	41.28	44.15	10.38	4.19	
Totals	n	285	314	82	32	713
	%	39.97	44.04	11.50	4.49	

TABLE 4. OVARIAN NEOPLASMS: RELATIVE FREQUENCY
IN PERSONS OF GROUP A COMPARED WITH FREQUENCY
OF ONE IN PERSONS OF GROUP O

Comparisons	Blood Group A	Blood Group O	Relative Frequency in Group A	χ^2	P
Benign	114	98	1.29	3.24	.07
Control	1,828	2,029			
Malignant	200	187	1.19	2.66	.10
Control	1,828	2,029			
Total	314	285	1.22	5.13	.025
Control	1,828	2,029			

by only four of the ten categories: pseudomucinous cysts, simple cysts, endometriosis, and dermoid cysts (table 7). There were only eleven simple cysts in these data and these are therefore excluded for the purpose of statistical comparisons. While impressive ratios occur in all three categories given, only in dermoids is the ratio (2.40) statistically significant, $P = .013$.

In the nine categories of malignant disease the excess of blood group A is contributed only by two: papillary adenocarcinoma and secondary carcinoma (table 8). The ratio of 1.44 for papillary adenocarcinoma is statistically significant, $P = .015$, as is the ratio of 6.10 for secondary carcinoma, $P = .017$.

With the exceptions of papillary adenocarcinoma and adenocarcinoma the number of cases in a given category are inadequate for statistical comparisons. The relative frequency of papillary adenocarcinoma compared with adenocarcinoma is 1.80 in persons of group A to one in persons of group O, $P = .022$. It would appear that the ABO blood groups may relate in some way to the presence of papillary features in an ovarian adenocarcinoma.

DISCUSSION

Since demonstration by Aird, Bentall and Roberts (1953) of the association between stomach cancer and blood group A, most subsequent blood group-cancer studies have been designed to test the proposition that blood group A is correlated with carcinogenesis by virtue of the fact that an established malignancy has been the customary basis for selection of patient or disease populations. Such a study design precludes the possibility of detecting a blood group

TABLE 5. BLOOD GROUP DISTRIBUTION IN DIFFERENT CLASSIFICATIONS OF BENIGN OVARIAN NEOPLASMS

Classification		O	A	B	AB	Total
Pseudomucinous	n	7	15	4	2	28
	%	25.00	53.57	14.29	7.14	
Mucinous	n	5	1	—	—	6
	%	83.33	16.67	—	—	
Simple cyst	n	4	5	2	—	11
	%	36.36	45.45	18.18	—	
Serous cyst	n	14	13	4	1	32
	%	43.75	40.63	12.50	3.13	
Endometrial cyst	n	6	5	2	—	13
	%	46.15	38.46	15.38	—	
Endometriosis	n	20	29	6	2	57
	%	35.09	50.88	10.53	3.51	
Dermoid	n	12	26	8	1	47
	%	25.53	55.32	17.02	2.13	
Teratoma	n	6	2	—	1	9
	%	66.67	22.22	—	11.11	
Fibroma	n	13	9	3	4	29
	%	44.83	31.03	10.34	13.79	
Thecoma & granulosa cell	n	11	5	4	—	20
	%	55.00	25.00	20.00	—	

TABLE 6. BLOOD GROUP DISTRIBUTION IN DIFFERENT CLASSIFICATIONS OF MALIGNANT OVARIAN NEOPLASMS

Classification		O	A	B	AB	Total
Pseudomucinous	n	8	8	—	1	17
	%	47.06	47.06	—	5.88	
Mucinous	n	7	4	4	—	15
	%	46.67	26.67	26.67	—	
Cystadenocarcinoma	n	5	2	—	2	9
	%	55.56	22.22	—	22.22	
Serous cystadenocarcinoma	n	6	6	2	—	14
	%	42.86	42.86	14.29	—	
Anaplastic adenocarcinoma	n	9	8	3	—	20
	%	45.00	40.00	15.00	—	
Adenocarcinoma	n	54	39	8	2	103
	%	52.43	37.86	7.77	1.94	
Papillary adenocarcinoma	n	84	109	28	13	234
	%	35.90	46.58	11.97	5.56	
Secondary carcinoma	n	2	11	1	—	14
	%	14.29	78.57	7.14	—	
Granulosa cell	n	7	4	1	—	12
	%	58.33	33.33	8.33	—	

TABLE 7. BENIGN OVARIAN NEOPLASMS: RELATIVE FREQUENCY IN PERSONS OF GROUP A COMPARED WITH FREQUENCY OF ONE IN PERSONS OF GROUP O

	Blood Group A	Blood Group O	Relative Frequency in Group A	χ^2	P
Pseudomucinous	15	7	2.38	3.57	.06
Control	1,828	2,029			
Endometriosis	29	20	1.61	2.65	.09
Control	1,828	2,029			
Dermoid cyst	26	12	2.40	6.24	.013
Control	1,828	2,029			

TABLE 8. MALIGNANT OVARIAN TUMORS: RELATIVE FREQUENCY IN PERSONS OF GROUP A COMPARED WITH FREQUENCY OF ONE IN PERSONS OF GROUP O

	Blood Group A	Blood Group O	Relative Frequency in Group A	χ^2	P
Papillary Adenocarcinoma	109	84	1.44	6.02	.015
Control	1,828	2,029			
Secondary Carcinoma	11	2	6.10	5.52	.017
Control	1,828	2,029			

association with a pre-malignant condition or other biological process which may be responsible for the increased cancer susceptibility observed for persons of blood group A. In the present study, as in the study of salivary gland tumors (Osborne and De George, 1962), benign as well as malignant disease has been included for the express purpose of determining whether some process other than carcinogenesis may be responsible for an observed blood group-cancer association.

In the 713 cases of ovarian disease analyzed in the present study there are 453 cases of malignant and 260 cases of benign disease. In women with blood group A, the frequency of ovarian carcinoma is 1.19 relative to one in women with blood group O, $P = .10$. While not statistically significant, these data are consistent with the German data reported by Helmbold (1961). In a series of 1,300 cases of ovarian carcinoma obtained from fourteen different patient populations, Helmbold found a frequency of ovarian carcinoma of 1.165 in women with blood group A relative to one in women of blood group O. In these larger data, $P = .018$.

The benign ovarian diseases in the present study have a frequency of 1.29 in women of blood group A relative to one in women of blood group O, $P = .07$. Though neither benign nor malignant disease associates significantly with blood group A when taken separately, both contribute to the significant ratio of 1.22, $P = .025$, obtained for the total series of 713 cases. It would appear that in the ovary, as in the salivary glands (Cameron, 1958; Osborne and De George, 1962), the blood group A association relates to benign as well as malignant neoplastic disease. It can therefore be concluded that the associations which have been demonstrated between blood group A and cancer at other anatomical sites, may also relate to some pre-malignant condition or other biological process rather than to carcinogenesis *per se*.

To extend the interpretation of these data nineteen different classifications of ovarian disease were made, and analyzed separately. It is found that only six classifications (four benign and two malignant) contribute to the increased frequency of ovarian disease in women of blood group A. These are: pseudomucinous cysts, endometriosis, dermoid cysts, simple cysts, papillary adenocarcinoma and secondary carcinoma. Some of the characteristics common to these ovarian diseases are of interest. Pseudomucinous cysts, in addition to their specific content in secretor women, are lined by a high columnar epithelium resembling the epithelium of the uterine cervix or large intestine (Anderson, 1948; Willis, 1960). The extra-ovarian and cystic characteristics of endometriosis and of dermoids are implicit. Papillary adenocarcinoma is distinguished from adenocarcinoma by the presence of papillary development or over-growth atypical of the adult ovarian epithelium by which the ovarian adenocarcinoma is defined. The possible significance of these papillary features to the blood group A association is strongly implied by a significant difference between these two varieties of carcinoma in the frequency of blood group A relative to that of blood group O, (ratio = 1.80, $P = .022$). Secondary carcinoma, which has a sixfold increase in the frequency of blood group A, is classified on the basis of the fact that the cell type of the malignant ovarian tissue is of another anatomical site,

principally gastrointestinal, mammary and cervical. In these secondary carcinomas, no other ovarian disease was described. Some secondary cases also occurred in which there was additional ovarian disease; these were classified as miscellaneous. When all such cases are reclassified as secondary carcinoma there are seventeen blood group A and four blood group O, giving a ratio of 4.72, $P = .005$.

Certain generalizations are possible from these data. The ovarian neoplasms which associate with blood group A are of a glandular type of epithelium, are of either a cystic or papillary structure, and include development of some atypical or extra-ovarian type of epithelium. In contrast, the ovarian diseases which do not appear to associate with blood group A are solid rather than cystic (Corscaden, 1956), and, if of an epithelial origin, they are entirely of an ovarian type.

The findings of this study are similar in all major respects to those of the parotid and submaxillary gland study. At both sites it was found that individuals of blood group A have a greater likelihood than do individuals of blood group O of developing benign as well as malignant disease, but only of limited histological types. These types are also similar at the two sites. In both the ovary and the parotid gland, tumors with papillary features associate with blood group A (papillary adenocarcinoma, adenolymphoma or papillary cystadenoma) while adenocarcinoma without papillary features is not associated with blood group A. As in the ovary, all types of salivary gland tumors with an increased frequency in individuals of blood group A (mixed tumors, mucoepidermoid tumors, squamous tumors, and adenolymphomas) possess some form of atypical or metaplastic epithelium (Evans, 1956; Frazell, 1954; Stewart, Foote and Beaker, 1945; Willis, 1960). In the salivary gland study, the potential importance of this characteristic to the blood group A association is most apparent in the mucinous tumors of the parotid gland, which arise by metaplasia (Willis, 1958). It was this observation which importantly lead to the suggestion that "the blood groups are associated with neoplastic processes which involve the mucous secreting elements of the glandular epithelium" (Osborne and De George, 1962). From the blood group A association with dermoid cysts of the ovary, it would appear that while the neoplastic processes which associate with the blood group A genotype may predominantly involve columnar mucous-secreting epithelium, it is the characteristic of atypical cell regeneration or metaplasia which is critical to this association rather than mucus secretion itself.

The reparative and regenerative capabilities of different tissues and organs vary greatly, and in all probability differ also between individuals. The epithelium in general, and quite markedly that of the salivary glands and ovary, is capable of extensive regeneration. While proliferating adult cells are most typically unipotential and accurately reproduce cells of a fixed type, certain environmental conditions such as inflammation or nutritional deficiencies may incline some proliferating cells to differentiate along a different or atypical pathway (Ham and Leeson, 1961). The salivary gland and ovarian epithelium is known to possess dormant potential for widely aberrant differentiation in regenerative or other proliferative lesions (Willis, 1958, and many others). Both the salivary

gland and ovarian disease data suggest that under conditions which may stimulate or demand epithelial regeneration at these two sites, different potentials for atypical cell differentiation or metaplasia may be associated with different ABO blood group genotypes. Such a thesis is equally compatible with the present evidence concerning the blood group associations with gastrointestinal disease; an investigation of this is now in progress.

The ovarian disease data, like those of the salivary gland study, unquestionably indicate that the increased cancer risk of individuals of blood group A is a consequence of a blood group A association with some pre-malignant process. The fact that a specific pre-malignant process may be involved is implied by the limitation of the blood group A association with only certain histological types of ovarian disease. The similarity in histological characteristics of the ovarian diseases and salivary gland tumors which associate with blood group A indicate the presence of some common denominator; this suggests that there may well be some common basis for all blood group disease associations.

SUMMARY

Neoplastic diseases of the ovary were investigated with respect to the ABO blood groups as a sequel to a study of salivary gland tumors in which it was found that blood group associations occurred with benign as well as with malignant tumors, but only with tumors of certain histological appearances. The findings for the blood group associations with ovarian disease are compatible with those of the salivary gland study in all major respects.

The ovarian disease data, like those of the salivary gland study, unquestionably indicate that the increased cancer risk of individuals of blood group A is a consequence of a blood group A association with some pre-malignant process. On the basis of the types of neoplastic diseases of the ovary and of the salivary gland which associate with blood group A, it is probable that different potentials for atypical cell differentiation may be associated with the different ABO blood group genotypes.

An unexpected finding which will require verification from further investigation, but one with great potential importance to the cancer problem, is the four to sixfold excess of secondary carcinomas of the ovary in women of blood group A relative to that in women of blood group O.

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Paternal Age Effect for Cleft Lip and Palate

CHARLES M. WOOLF¹

Arizona State University, Tempe, Arizona

A GENETIC STUDY of cleft lip and palate was carried out in Utah by Woolf, Woolf and Broadbent (1963a). The results support the conclusions of others (Fogh-Anderson, 1942; Fraser, 1955) that cleft lip with or without cleft palate, symbolized by CL(P), is genetically different from isolated cleft palate (CP). Although the propiiti used in this genetic study were not collected primarily for this reason, the ages of their mothers were analyzed to determine if a parental age effect was evident. Maternal age was used as the measurement of parental age since comparative vital statistics data are readily available. A slight but statistically significant positive relationship was found between maternal age and CL(P), but not CP (Woolf, Woolf and Broadbent, 1963b). This supports the conclusion of MacMahon and McKeown (1953). Other investigators have presented data showing a significant relationship between maternal age and congenital clefts of the lip and palate (Phair, 1947; Loretz, Westmoreland and Richards, 1961). Fraser and Calnan (1961) have concluded that paternal age is more important than maternal age.

The present paper summarizes the results of a further study designed to determine if the parental age effect observed for the propiiti with CL(P) is of paternal or maternal origin.

The CL(P) propiiti were ascertained from surgical records in Utah. The ages of the parents at the birth of the propiiti were obtained from medical records or at the time family members were interviewed. Ages of the parents were available for 411 of 418 propiiti. As a control group, parental ages were obtained for 411 births occurring in the state of Utah during the years 1953-1960. Birth certificates were made available through the courtesy of Mr. John W. Wright, Director of the Bureau of Vital Statistics, Utah State Department of Public Health. The books containing the birth certificates for these years were opened randomly and the ages of the parents recorded. The control group may not be completely appropriate since the propiiti were variable in age and were not all born in Utah during this period of time. However, this control group was used in the absence of a more suitable one.

The parental age data were placed in frequency distribution tables with the following classes: under 19, 20-24, 25-29, 30-34, 35-39, and over 40. A comparison of maternal ages is given in table 1. As compared with the control group, there is a deficiency of CL(P) propiiti born to younger mothers and an excess born to older mothers. The chi square value calculated from this 6 x 2 contingency table (5 degrees of freedom) is highly significant ($\chi^2 = 16.9$;

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¹Present address: Department of Genetics, University of Utah, Salt Lake City, Utah.

$P < 0.01$). Table 2 shows a similar comparison of paternal ages. The chi square value is also highly significant ($\chi^2 = 18.7$; $P < 0.01$).

Two covariance analyses were then carried out to test the hypothesis that the adjusted mean ages of the parents of the CL(P) propiiti do not exceed those for the control groups. In the first analysis (table 3), maternal adjusted mean ages were compared. In the second analysis (table 4), paternal adjusted mean ages were compared. The probability of rejecting the hypothesis if it is indeed true (Type I error) was set at 0.05 in each analysis. On the basis of the F tests, the hypothesis should be accepted in the first analysis and rejected in the second analysis.

The group means before adjustment, adjusted group means, and differences between these means are given in table 5. Before adjustment, the mean ages of the fathers and mothers of the propiiti are significantly higher than the mean ages of the respective control groups. After adjustment, the father's mean age is

TABLE 1. A COMPARISON OF THE AGES OF THE MOTHERS OF CL(P) PROPOSITI WITH CONTROLS

Age	Mothers of Propiiti	Maternal Controls	Deviation
Under 19	22	52	-30
20-24	123	127	-4
25-29	119	101	+18
30-34	80	76	+4
35-39	49	46	+3
40 and over	18	9	+9
Total	411	411	

TABLE 2. A COMPARISON OF THE AGES OF THE FATHERS OF CL(P) PROPOSITI WITH CONTROLS

Age	Fathers of Propiiti	Paternal Controls	Deviation
Under 19	4	8	-4
20-24	63	105	-42
25-29	135	118	+17
30-34	86	91	-5
35-39	74	56	+18
40 and over	49	33	+16
Total	411	411	

TABLE 3. COVARIANCE ANALYSIS. COMPARISON OF MOTHER'S AGES WHEN ADJUSTMENT IS MADE FOR FATHER'S AGES

Source of Variation	Deviations About Regression		
	Degrees of Freedom	Sum of Squares	Mean Square
Total	820	8,899.77	—
Within Groups	819	8,899.10	10.87
Difference for Testing Adjusted Group Means	1	0.67	0.67
$F = 0.67/10.87 = 0.06$			

TABLE 4. COVARIANCE ANALYSIS. COMPARISON OF FATHER'S AGES WHEN ADJUSTMENT IS MADE FOR MOTHER'S AGES

Source of Variation	Deviations About Regression		
	Degrees of Freedom	Sum of Squares	Mean Square
Total	820	11,071.92	—
Within Groups Means	819	11,024.78	13.46
Difference for Testing Adjusted Group Means	1	47.14	47.14*
F = 47.14/13.46 = 3.50			

*Significant at 0.05 level

TABLE 5. A COMPARISON GROUP OF MEANS BEFORE AND AFTER ADJUSTMENT

Hypothesis: $\mu_1 \leq \mu_2$ Probability of a Type I error: 0.05						
Mothers of Propositi (\bar{y}_1)	Maternal Controls (\bar{y}_2)	Fathers of Propositi (\bar{y}_1)	Paternal Controls (\bar{y}_2)	Difference $\bar{y}_1 - \bar{y}_2^*$	Conclusions	
27.7	26.6	—	—	+1.1*	Reject hypothesis	Reject hypothesis
—	—	30.9	29.4	+1.5*	Reject hypothesis	Reject hypothesis
27.1	27.2	—	—	—0.1	Accept hypothesis	Accept hypothesis
—	—	30.4	29.9	+0.5*	Reject hypothesis	Reject hypothesis

*t > +1.64

still significantly higher, but the mother's mean age is actually lower than the control mean. It is concluded from these analyses that a paternal age effect exists for this anomaly.

DISCUSSION

Clefts can be induced in experimental animals by teratogenic agents (see Fraser, 1962). This observation plus the genetic data (Fraser, 1955; Woolf, Woolf and Broadbent, 1963a) suggests that clefts may be a manifestation of multiple genetic and non-genetic factors disturbing development. Evidence that at least one type of chromosomal abnormality results in cleft lip and palate is the occurrence of these anomalies in some individuals with Trisomy 13-15 syndrome (Lubs, Koenig and Brandt, 1961). Chromosomal aberrations may account for other cases.

Penrose (1962) has demonstrated a paternal age effect for the 21/22 type of Down's syndrome. He proposes that this is due to differential gametic selection with advancing paternal age. Relaxation of selection with advancing paternal age against those gametes containing a genetic or chromosomal mechanism predisposing to CL(P) might also explain the slight paternal age effect noted in this study.

Although differential gametic selection with increasing paternal age is an intriguing hypothesis, alternative hypotheses should be considered as well, such as differential mutation rate or accumulation of mutations with advancing paternal age. At the present, it is not possible to discriminate among these hypotheses.

SUMMARY

1. The ages of the parents at the birth of 411 probands with cleft lip with or without cleft palate were compared with the ages of parents selected randomly from birth certificates. A parental age effect was demonstrated for this congenital anomaly. The risk of producing a child with this disorder is decreased in younger parents and increased in older parents.
2. Covariance analyses have shown that paternal age is of etiological importance.
3. Differential gametic selection with advancing paternal age is one explanation for the paternal age effect. However, differential mutation rate and accumulation of mutations with advancing paternal age should also be considered.

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Linkage of the β -Chain and δ -Chain Structural Genes of Human Hemoglobins

BENNETT F. HORTON AND TITUS H. J. HUISMAN

*Division of Hematology, Department of Medicine,
and the
Departments of Pathology and Biochemistry,
Medical College of Georgia, Augusta, Georgia*

EVIDENCE IS ACCUMULATING that the genetic mechanism controlling the formation of human hemoglobins consists of four structural genes and an unknown number of controller genes (Neel, 1961; Motulsky, 1962). Of the four structural genes which direct the amino acid sequences of the four polypeptide chains, termed α , β , γ and δ -chains, the β gene and the δ gene, and perhaps the γ gene seem to be closely linked, while the α gene appears not to be closely linked to the other structural genes (Neel, 1961).

The evidence for the linkage of the β gene and the δ gene consists of the segregation in several families of abnormal δ genes and an abnormal β gene. This communication reports briefly an additional family in which an interaction of Hb-S (a β -chain abnormality) and of Hb-A₂' (a δ -chain abnormality also sometimes designated Hb-B₂) occurs and summarizes the data reported to date indicating close linkage of the β -chain and the δ -chain structural genes.

METHODS

Starch gel electrophoresis was used to establish the hemoglobin patterns (Huisman, 1960). The presence of Hb-S was confirmed by determining the solubility of the reduced hemoglobin according to the method of Itano (1953). The percentages of the different hemoglobin components were determined by DEAE cellulose chromatography (Huisman and Dozy, 1962).

RESULTS AND DISCUSSION

The pedigree of the newly discovered family (family H) is presented in Fig. 1. The proband, a Negro male 22 years of age who had no children, was heterozygous for both Hb-S and Hb-A₂'. Of his six sisters five were available for further study. Three sisters were found to be heterozygous for Hb-S, one heterozygous for Hb-A₂', while one was doubly heterozygous for Hb-S and Hb-A₂'. This doubly heterozygous sister had three children by two unavailable husbands and five by a husband with a normal hemoglobin pattern. Four of the eight children were found to be heterozygous for Hb-S and four heterozygous for Hb-A₂'. Quantitative analysis of the hemoglobin fractions in the blood of the Hb-A₂' heterozygous individuals revealed approximately half of the usual

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per cent of Hb-A₂ (0.8 to 1.3 per cent) with percentages of Hb-A₂' of 0.7 to 1.2 per cent, the total quantities of δ -chain containing hemoglobin types being similar to that found in normal individuals.

Table 1 summarizes the inheritance patterns of the β and δ genes as seen in the children of a parent doubly heterozygous for Hb-S and a δ -chain abnormality as reported to date in the literature. In many descendents this pattern is definite, while in others some question of the pattern exists, either because of an unknown hemoglobin pattern of the other parent or because of the presence of an abnormal hemoglobin gene in this parent. When data related only to the 21 children with a definite inheritance pattern were taken into account, it was found that thirteen were heterozygous for the β -chain abnormality, eight heterozygous for the δ -chain abnormality, while no normal or doubly heterozy-

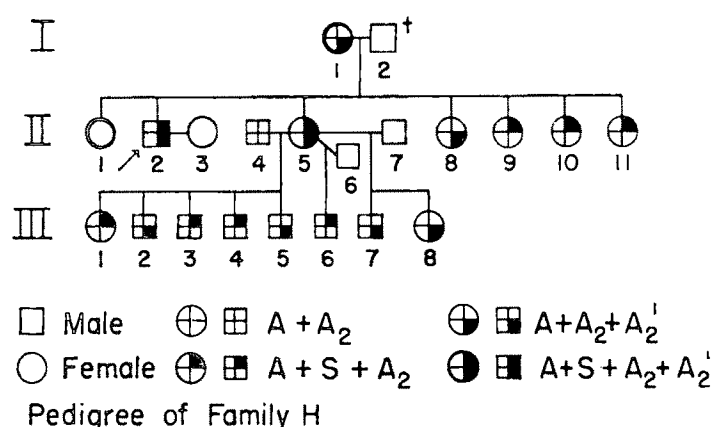


TABLE 1. SUMMARY OF DATA REPORTED TO DATE ON GENETIC LINKAGE OF THE LOCI OF THE β -CHAIN AND THE δ -CHAIN OF HUMAN HEMOGLOBINS

Family	Reference	Gene Patterns Transmitted From a Double Heterozygote							
		$\beta_2^A \delta_2^{A^2}$		$\beta_2^S \delta_2^{A^2}$		$\beta_2^A \delta_2^{Abn.}$		$\beta_2^S \delta_2^{Abn.}$	
—	Cepellini (1959)	*	†	*	†	*	†	*	†
R	Horton <i>et al.</i> (1961)	0	(0)	4	(4)	2	(2)	0	(0)
Flatbush (A) ‡	Ranney <i>et al.</i> (1963)	0	(0)	1	(1)	0	(4)	0	(0)
Flatbush (B) ‡	Ranney <i>et al.</i> (1963)	0	(0)	2	(2)	3	(3)	0	(0)
Flatbush (C) ‡	Ranney <i>et al.</i> (1963)	0	(0)	0	(2)	0	(1)	0	(0)
H	This paper	0	(0)	3	(3)	1	(1)	0	(0)
TOTAL		0	(0)	13	(16)	8	(15)	0	(0)

*Data of genetic transmission patterns which are beyond reasonable doubt. The genes were received from a parent with a double heterozygosity, who married a normal individual or an individual whose gene transmission could be determined.

†Data include those examples where the genetic transmission was probable but where the genes mentioned might have come from the other parent.

‡Family Flatbush (A) refers to the descendents of the cases II-5 and II-6, Flatbush (B) to the descendents of the cases II-7, II-8, and II-9 and Flatbush (C) to the descendents of the cases II-15, II-16 and II-17 as presented in the original pedigree of this family (Ranney *et al.*, 1963).

gous children have been reported. The statistical probability of linkage of the β and δ genes as calculated by the fourfold table, utilizing the correction formula of Yates because of the small number of cases, is great; the chi square is 8.5 corresponding to a p value < 0.01 (Hill, 1955).

In classical genetic terms the closeness of linkage is measured in frequency of cross-overs. With cases reported in this communication included, there are 21 well documented opportunities for a cross-over of these two loci, while no cross-over has been detected. In addition to these 21 cases, 10 other cases have been found in which an opportunity for a cross-over was present without any being obvious. In these 10 cases, however, cross-overs cannot categorically be excluded because of unknown or abnormal genes in the other parent. At first sight no great objections are apparent for including the descendents of an assumed "normal" parent in the calculation of frequency of cross-overs, particularly when the descendents carry only a rare abnormal gene such as a δ -chain abnormality, which is unlikely to come from a random parent. On the other hand, the authors have had an experience with a family in which the presence of a rare blood group variant in both the reputed father and son would argue strongly for paternity, whereas another blood type excluded this possibility (Thompson *et al.*, 1961). The extensiveness of the families reported, as well as the recorded first cousin marriage in one of the families, would point out that even a rare gene might be quite easily available from an uninvestigated parent in a particular situation where this gene has been proved to be present in the area.

SUMMARY

A family with an interaction of Hb-S, a β -chain abnormality, and Hb-A₂', a δ -chain abnormality, is reported. From these and previously reported data pertaining to 21 descendents of four families, in which the inheritance pattern is beyond reasonable doubt, it was concluded that the probability of these two loci being closely linked is significant (p value < 0.01).

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The Study of Normal Variation in Man

I. Interrelations of Adiposity, Ancestry, and Blood Type

HERMAN M. SLATIS¹ AND ASHER J. FINKEL

*Division of Biological and Medical Research and Health Division,
Argonne National Laboratory, Argonne, Illinois*

THIS STUDY WAS INITIATED after Angel (1957) reported an elevated frequency of blood groups B and AB in a small sample of obese persons. Angel noted that a number of hypotheses might explain this relationship and, without being told that our suspicions lay in this direction, added (1960) that there was not an excess of persons of East European ancestry among the obese. Mathew (1959) failed to find statistically significant relationships between ABO blood groups and weight or blood pressure in a large sample of soldiers in the Indian Army. However, he did find a significant association between blood group and height, and he further showed that this was general among the various ethnic and geographic subgroups that comprised the sample. Mathew suggested that the B allele might contribute pleiotropically to increased height. He also suggested close linkage of B and a gene for increased height, which, on the whole, is an untenable hypothesis for such phenomena. Mathew further noted the low likelihood that the Indian population is composed of the descendants of a tall group with high B frequency and a short group with low B frequency and that reassortment of the genes is not yet complete. Linder, Weber and Morgenthaler (1957) failed to find a relationship between blood group and a number of anthropometric measurements including height in a small sample of Swiss military recruits. With their data, discriminant analysis was not useful as a tool for distinguishing men of different blood groups.

The present study was set up to test the independence of height, weight, and blood type in a U. S. population composed largely of unrelated persons of a variety of ancestries.

THE POPULATION

Almost all persons undergoing routine medical examinations at Argonne National Laboratory (ANL) during 1959 and the first part of 1960 were included in this study. Data obtained on 3,709 persons included: sex, date of birth, ABO blood type (anti-A, anti-B), Rh blood type (anti-D), height (to nearest inch, stocking feet), weight (to nearest pound, normal indoor clothing less

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¹Present address: Department of Zoology, Michigan State University, East Lansing, Michigan.

jacket and shoes), birthplace of each parent, and national origin of each grandparent.

Certain arbitrary standardizations were made in describing ancestry. All persons clearly having some Negro ancestry were classified as Negroes. "Old Americans" were classified as British, which undoubtedly describes the origin of the bulk of the genes involved. Persons who are at least three-quarters of a given ancestry were placed with those entirely of that ancestry, while persons of more mixed ancestry were classified according to their mixture. Ancestry was classified by country or geographical area rather than by subgroups within the various countries. Of importance are that (1) Denmark, Norway and Sweden will be referred to as Scandinavia, (2) Germany, Austria and Switzerland will be referred to as Germany, and (3) Poland, Lithuania, Latvia, and Russia will be referred to as East Europe.

RELATIVE WEIGHTS

A study of an American sample by the Society of Actuaries (SA) (1959) was used to determine the relationship between height, age, and weight for each sex. The ANL population differs from that of the SA study in that the ANL men are about nine pounds heavier, while the ANL women are about two pounds heavier. Each person in our study was assigned a "relative weight," which is the number of pounds by which that person differs from the mean of persons of the same height-age group in the SA study, after correction for the ANL-SA difference. Therefore, the mean relative weight is always zero. Seven men and three women who were outside of the height range of the SA tables were not assigned relative weights. Therefore, data concerning relative weights are based on 3,000 men and 699 women. All other data are based on 3,007 men and 702 women.

ANALYSIS OF THE DATA

For each sex, the data can be grouped into five categories: (1) height, (2) relative weight, (3) month and year of birth, (4) place of birth, place of parents' birth, and ancestry, and (5) blood type. There are ten simple relationships between these five items of data, and, where they are of interest, these relationships will be taken up individually.

1. *Height Versus Month and Year of Birth*

There is a clear relationship between height and year of birth. The least squares line of best fit for the relationship of height to year of birth among the men indicates an increase of $.055 \pm .007$ inches per year. The existence of this phenomenon of increased height among more recently-born men was observed at least 60 years ago (*e.g.*, Pearson and Lee, 1903). The women show almost the same rate of increase in height by year of birth as that shown by the men, $.053 \pm .009$ inches per year. Both environmental and genetic reasons might be adduced for this shift of height with time, and this is discussed in the next section.

Month of birth may be related to adult height. The average height of winter-born men (December through March) is greater than that of men born in any other month (table 1). If only the men less than 40 years old are considered (62 per cent of the male sample), the data come closer to the form of a sine curve, which is what one would expect of a characteristic that shows seasonal variation. The winter-born men less than 40 years old are .34 inches taller than summer-born men. While this difference is 2.86 times its standard error and has a probability of less than .01, the true probability of this result is undoubtedly not this small because of the *a posteriori* nature of this analysis.

In contrast with the men, women born in each month from September through December have a mean height shorter than women born in any of the other eight months (table 1). Further analysis does not indicate that any subgroup among these women shows the seasonal variation more clearly than the group as a whole, except that among the women less than 40 years old (74 per cent of the female sample), the mean height in each month from July through December (except September) is less than the mean height in any of the first six months of the year.

2. Height Versus Place of Birth and Ancestry

Both place of birth and ancestry have distinct relationships with height. Foreign-born men show an increase in height by year of birth of $.091 \pm .018$ inches per year, while U. S.-born men show only $.049 \pm .007$ inches per year. Similar differences are found among the women. The youngest men of both groups are of almost precisely the same average height, but there is a great divergence in mean height among older men of these two groups. Differences in ancestry do not appear to be responsible for the shift in height.

Among the U. S.-born men a definite genetic phenomenon, hybrid vigor, might be suggested as the mechanism responsible for the shift in height with year of birth, since the breakdown of isolates increases with time. Table 2 indicates the major nationality groups that are present in this sample and those groups of mixed ancestry that number at least ten men. The list is by increasing

TABLE 1. MEAN HEIGHT BY MONTH OF BIRTH

	All Men	Men under Age 40	All Women	Women under Age 40
January	69.18	69.65	64.57	64.92
February	69.63	69.72	64.49	64.80
March	69.19	69.67	64.29	64.67
April	69.03	69.42	64.45	64.76
May	69.08	69.22	65.15	65.33
June	69.01	69.35	64.33	64.89
July	69.15	69.22	64.32	64.33
August	68.80	69.16	64.29	64.60
September	68.97	69.35	64.21	64.83
October	69.14	69.75	63.71	64.15
November	68.88	69.49	63.61	63.73
December	69.22	69.54	64.07	64.50
Minimum sample size	225	134	39	33
Maximum sample size	272	174	70	56
Minimum standard error	.15	.19	.24	.26
Maximum standard error	.19	.23	.44	.45

TABLE 2. DISTRIBUTION OF VARIOUS CHARACTERISTICS OF MAJOR NATIONALITY GROUPS AND OF HYBRIDS
BETWEEN THEM IN THE ANL POPULATION OF MEN

Group	Mean Relative Weight	Variance of Weight	Mean Height	Variance of Height	Number Observed	Mean Year of Birth	Gene B Frequency
British	-4.27	459	69.03	6.51	624	1920.20	.07
Negro	-2.52	782	68.28	8.46	95 (1)*	1918.97	.10
Czechoslovakian	1.11	449	69.45	5.51	144	1921.33	.13
German	1.13	480	69.38	7.11	441 (1)	1920.99	.09
Scandinavian	1.27	417	69.74	6.69	134 (1)	1921.50	.07
Italian	2.55	446	67.69	8.16	110	1923.06	.06
East European	5.45	576	69.00	7.62	384 (1)	1921.72	.15
Brit./Czech.	-3.78	435	69.43	4.72	14	1926.21	.11
Czech./E. Eur.	-3.07	1250	70.43	12.42	14	1926.71	.17
Brit./E. Eur.	-1.49	351	69.07	3.61	14	1927.64	.11
Brit./Scand.	-.55	358	69.95	6.18	60	1924.15	.09
Ger./Scand.	-.26	718	70.27	7.33	33	1923.94	.12
Czech./Ger.	.08	797	68.57	4.48	28	1923.14	.08
Brit./Ger.	.33	681	69.32	7.57	247 (3)	1922.53	.05
E. Eur./Ger.	.68	646	69.44	8.14	55	1924.02	.09

*Numbers in parentheses indicate the number of persons not assigned a relative weight.

relative weight. Of the eight mixed-ancestry groups, seven have mean heights greater than the mean of the two groups from which they are descended, and this might be interpreted as an indication of hybrid vigor.

After appropriate correction for mean year and country of birth, the distribution of the groups of mixed ancestry is close to that expected from the data for men of single-ancestry groups. Because men of recent birth have achieved about the same mean height whether born in the U. S. or in Europe, an environmental, rather than a genetic cause for the increased height of young men is suggested. Height has roughly the same variance for all groups, ranging from about 5.5 inches to 8.4 inches both in the larger mixed-ancestry and single-ancestry groups.

Of course, even U.S.-born men of the various ancestry groups differ significantly from one another in their mean heights. This fact is not important to the present study.

3. *Height Versus Blood Type*

The population can be divided into eight categories according to Rh and ABO blood types (table 3). In both sexes, an analysis of variance of height for these eight categories shows no significance for Rh or ABO differences, or for the interaction between them. Mathew (1959) reported that men of type B average 0.6 cm. taller than other men. Our study confirms this with a difference of 0.20 inches (0.5 cm). Mathew observed a population with a larger proportion of gene B among half again as many men as were seen in the ANL study. Consequently, a similar difference in height proved to be highly significant in Mathew's population and not significant in ours. Since the difference is small and the variance of height within each blood type is roughly identical, a high frequency of tall type B men is not expected.

Women of blood type B were slightly shorter than the mean height of all women (table 3). Consequently, there is no evidence that gene B contributes to increased height among women.

4. *Relative Weight Versus Place of Birth and Ancestry*

Both ancestry and place of birth appear to have effects on relative weight. Among the men, there is a general increase in weight as ancestry shifts eastward across Europe, with British the lightest in weight, Scandinavians, Germans, Czechoslovaks, and Italians intermediate, and East Europeans the heaviest. This array of differences (table 2) is highly significant. The only large non-European group in this sample, Negroes, falls within the weight range of the white groups. The Japanese sample (ten men, nine of whom are of U. S. birth) is an extreme example of the differences between groups. Its mean relative weight (corrected for shorter mean stature) is more than 25 pounds below that of the total ANL population.

In contrast to the situation with height, men of mixed ancestry tend to have a higher variance of relative weight (about 700 pounds) than do men of single ancestry (about 500 pounds). The 94 Negroes, who really constitute a mixed-ancestry group between African Negroes and British, have a variance in the

TABLE 3. MEAN HEIGHT AND MEAN RELATIVE WEIGHT AND THEIR STANDARD ERRORS
AMONG PERSONS OF VARIOUS BLOOD GROUPS

Blood group		O			A		B		AB		Total
Rh +	Height	69.00 ± 0.08	69.15 ± 0.08	69.36 ± 0.15	69.04 ± 0.26	69.11 ± 0.05	1039 (4)*	1042 (1)	317	105	2503 (5)
	Rel. Weight	-0.92 ± 0.69	0.61 ± 0.74	2.04 ± 1.33	-1.36 ± 2.14	0.07 ± 0.46					
	No.										
Rh -	Height	69.23 ± 0.20	68.97 ± 0.19	69.01 ± 0.33	69.54 ± 0.52	69.12 ± 0.12	1039 (4)*	1042 (1)	317	105	2503 (5)
	Rel. Weight	-1.76 ± 1.48	-0.42 ± 1.89	3.85 ± 2.19	0.11 ± 3.58	-0.36 ± 1.02					
	No.	217 (2)	188	71	28	504 (2)					
Total	Height	69.04 ± 0.08	69.13 ± 0.08	69.29 ± 0.14	69.14 ± 0.23	69.11 ± 0.05	1256 (6)	1230 (1)	388	133	3007 (7)
	Rel. Weight	-1.07 ± 0.63	0.45 ± 0.69	2.37 ± 1.16	-1.05 ± 1.84	0.00 ± 0.42					
	No.										
Rh +	Height	64.14 ± 0.17	64.34 ± 0.16	64.16 ± 0.29	64.85 ± 0.53	64.25 ± 0.10	242 (1)	261 (2)	64	20	587 (3)
	Rel. Weight	0.92 ± 1.30	-0.66 ± 1.17	-1.10 ± 2.46	1.55 ± 3.87	0.02 ± 0.80					
	No.										
Rh -	Height	64.11 ± 0.39	64.60 ± 0.37	64.13 ± 0.80	64.60 ± 0.81	64.34 ± 0.25	47	48	15	5	115
	Rel. Weight	-0.87 ± 2.55	-0.47 ± 3.69	2.04 ± 5.52	4.30 ± 17.06	-0.10 ± 2.09					
	No.										
Total	Height	64.13 ± 0.15	64.38 ± 0.14	64.15 ± 0.28	64.80 ± 0.44	64.27 ± 0.10	289 (1)	309 (2)	79	25	702 (3)
	Rel. Weight	0.63 ± 1.17	-0.63 ± 1.14	-0.51 ± 2.24	2.10 ± 4.39	0.00 ± 0.75					
	No.										

*Numbers in parentheses indicate the number of persons not assigned a relative weight.

mixed range. Except for the Negroes, most of the persons of mixed ancestry are of the F_1 generation.

The place of one's birth and of the birth of one's parents has a highly significant effect on adult weight. (1) Men with both parents born in the U. S. have an average relative weight of -1.01 pounds; (2) those with one parent born abroad are somewhat heavier at $+0.41$ pounds (with little difference whether the father or mother was the foreign-born parent); (3) those with both parents born abroad average $+3.22$ pounds; (4) the foreign-born men are lightest, with a mean relative weight of -2.22 pounds. An analysis of variance of these four groups is highly significant ($F = 4.44$, the 1 per cent level of significance is at 3.34). However, the frequency of foreign birth or foreign-born parentage is non-random with respect to ancestry. Only the East European group contains moderate numbers of men in each birthplace category. These men show about the same distribution of weights according to place of birth as is shown by the ANL population as a whole, but the numbers are small and these differences are not significant. These relationships between weight and ancestry cannot be demonstrated in the small sample of women.

5. Relative Weight Versus Blood Type

Except for men of type B, the mean relative weight for any blood type is close to zero (table 3). An analysis of variance shows that the ABO variance is almost significant ($F = 2.50$, the 5 per cent level of significance is at 2.61). Neither the Rh type nor the Rh-ABO combinations show a statistically significant relationship with relative weight. In this sample, men of blood type B have the greatest mean weight, as was suggested by Angel (1957), so the near-significance of the findings merits further study.

The East European group is characterized by high relative weight and a high frequency of blood type B. Moreover, within that ancestry group, the type B men are almost four pounds above the group average (table 4). Analysis of the East European data shows a statistically significant difference in mean weight according to ABO blood group ($F = 3.07$, the 5 per cent level of significance is at 2.62, the 1 per cent level is at about 3.82).

Of the 102 men whose relative weight is at least 25 per cent above their expected weight, 18 are of type B and two of type AB (18 and 2 per cent, respectively, compared with 13 and 4 per cent B and AB in the male population as a whole). This does not confirm Angel's suggestion of a striking increase of B and AB among the obese.

TABLE 4. MEAN RELATIVE WEIGHT AMONG MEN
OF EAST EUROPEAN ANCESTRY

Blood group		O	A	B	AB	Total
Rh+	Rel. Weight	3.79	5.16	8.06	-6.62	4.60
	No.	174	210	107	31	522
Rh—	Rel. Weight	-1.59	-0.49	5.07	3.29	0.25
	No.	44	43	19	7	113
Total	Rel. Weight	2.71	4.20	7.61	-4.79	3.82
	No.	218	253	126	38	635

The small sample of women makes it difficult to observe minor shifts in weight related to blood type (table 3). However, in this sample the women of blood type B average among the lightest in weight, although the AB women were the heaviest. Both B and AB women were under-represented among the most obese women. There was no evidence of an unusual distribution of weights among O or A women or of a relationship between Rh blood type and weight.

6. *Blood Type Versus Ancestry*

That there is a strong correlation between blood type and ancestry is well known. In this study, each group that was represented by moderately large numbers of individuals had frequencies of ABO and Rh types compatible with those expected on the basis of previously published reports (as compiled by Mourant, Kopec, and Domaniewska-Sobczak, 1958).

There is no reason to believe that large scale errors of reporting of ancestry occurred. The gene frequencies for the British group are the most likely to reflect such errors because all persons claiming to be of "Old American" stock were added to those who knew that they were of British ancestry. There is no evidence that the British frequencies were shifted toward those of the continent through the addition of a continental genetic component by this arbitrary combination.

7. *Other Interrelationships*

No other relationships of interest were observed. The ANL and SA populations were similar in weight so that there was only a small relationship between relative weight and height or age. Season of birth did not show a relationship with weight, and neither season nor year of birth appear to be related to blood type.

DISCUSSION

This study has sought possible relationships between height, weight, blood type, month and year of birth, and birthplace and ancestry. Certain of the interrelationships between these characteristics have long been known. While no major new findings have been made, the data have enabled us to investigate a number of minor points.

It has been reported (Mathew, 1959) that men of blood type B are slightly taller than men of the other blood types. This finding has also been observed here, although our results were not statistically significant. Some evidence has been brought forth which suggests that height is slightly influenced by month of birth, and that this effect differs between the two sexes. No suggestion is advanced to account for a possible influence of month of birth on height and the relationship may be spurious.

A report by Angel (1957) suggested that persons of blood types B and AB were extremely frequent in a sample of obese individuals. Our study has found a slight shift toward higher weight among men of blood type B, but not AB, in relation to O and A, without an increase in variability. Such a shift barely

increases the frequency of obese B individuals. It is suggested that Angel's observations were due to an accident of sampling in a small population. Among the women there were smaller differences between the mean weights of the various blood types, and those ABO groups that were above average among the men were below average among the women, and vice versa.

Several factors that are associated with weight and blood type could make it appear that they are related. In our sample, persons of East European ancestry tend to be heavier, have a higher frequency of blood type B, tend to have foreign-born parents, and, perhaps, are more variable in weight than persons derived from the rest of Europe. Therefore, a large population of the kind studied in this report might show a correlation between weight and blood type because of factors that are correlated with ancestry, though many of these factors may be environmental in origin.

Some of the individuals in this study are descended from two nationality groups. The breakdown of nationality groups and the formation of persons of mixed ancestry is an ongoing event, in consequence of which, these persons tend to be younger than individuals of a single ancestry. Since age is related to factors such as adult height (which, in turn, is related to variability), there is some difficulty in assessing the effects of this crossing on the population. Correction for mean year of birth appears to distribute the height of persons of mixed ancestry around the mean of the heights of the two groups from which they came. Similarly, the weights of the individuals of mixed ancestry are close to the average weight of the appropriate ancestry groups. However, the mixed-ancestry groups seem to show a greater variability in weight than that shown by the single-ancestry groups. Various theories could be advanced to account for these observations. For example, both the British and German populations may be divided by religion into subpopulations that differ ethnically from each other, and positive assortment by religion might reinforce these biological differences.

SUMMARY

A study of 3,007 men and 702 women has failed to find any striking relationships between height, weight, and ABO or Rh blood type in a U.S. population. There appears to be confirmation for an earlier report that type B men are about .5 cm. taller than other men. There is little evidence for an increased frequency of type B among obese individuals. Month of birth may have a slight effect on adult height. Crossing between two groups of European ancestry does not appear to have an effect on mean height or weight, but the variance of weight may be increased among the men of mixed ancestry. Blood type, height, weight, and parental birthplace are correlated with ancestry, and in a large sample, these interrelationships might produce spurious correlations between blood type and height or weight.

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Genetic Heterogeneity in Human Acatalasia

HOWARD B. HAMILTON¹ AND JAMES V. NEEL²

WITH THE TECHNICAL ASSISTANCE OF KYOKO OZAKI¹ AND
TAKAJI NISHIZAWA¹

¹*Department of Clinical Laboratories of the Atomic Bomb Casualty Commission,
Hiroshima and Nagasaki, Japan, and*

²*Department of Human Genetics, University of Michigan Medical School,
Ann Arbor, Michigan, U.S.A.*

A SIGNIFICANT RECENT TREND in the study of the many inherited biochemical defects now known in man has been the demonstration, in a number of instances, that what were once considered as single entities are in fact genetically heterogeneous. This communication will submit evidence for the heterogeneity of still another biochemical trait, acatalasia, an inherited deficiency of the enzyme catalase, originally recognized among the Japanese (Takahara and Miyamoto, 1948) and later in Koreans (Yata, 1959) and Swiss (Aebi *et al.*, 1961). Studies in 1958 *et seq.* of catalase activity in the kindreds of several acatalasics showed a trimodal distribution of assay values, resulting in a clear distinction between normocatalasics or non-affected homozygotes, hypocatalasics or presumed heterozygous carriers, and acatalasics or affected homozygotes, with no overlap between the three groups (Nishimura *et al.*, 1959; Takahara *et al.*, 1960). These investigations thus confirmed and extended the initial hypothesis that this form of actalasia was transmitted by an autosomal "recessive" gene (Takahara *et al.*, 1952), the extension being the recognition of a clearly defined carrier state. We can now describe a kindred in which a gene responsible for acatalasia is segregating but where the heterozygous manifestations, clearly unlike those heretofore reported, are characterized by a considerable overlap with normal values. Two different approaches to the problem of accurately defining carrier values under these circumstances will be explored.

METHODS

The family was ascertained through an 18-year-old female. When, during a minor surgical procedure for chronic sinusitis, hydrogen peroxide was applied to the operative field, foaming failed to occur and the area turned black; in practice this has proved to be a good indication that catalase was absent. The operation was performed by Dr. M. Matsushima of the Saeki Hospital, Hat-

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sukaichi, Hiroshima Prefecture, and Dr. T. Yamashita of the ENT Service of the Medical Faculty of Hiroshima University, Hiroshima, Japan. The authors are indebted to Drs. Matsushima and Yamashita for bringing this large kindred to their attention and are grateful to Dr. Yamashita for his help in the early part of the field investigation.

Investigation of erythrocyte catalase activity was undertaken to obtain sufficient data from the immediate family to confirm the usual genetic pattern, but when several unexpected assay values were obtained it became apparent that a larger study was in order. Many members of the extensive sibship reside in one of three more or less contiguous farming areas west of Hiroshima City in Central Japan. Most of the others live in the city or neighboring suburbs. The field investigation was performed by one of two trained laboratory personnel who interviewed all family members for pertinent genealogical data and obtained approximately 5 ml of heparinized blood which was transported as rapidly as possible to the laboratory for ABO, MN, and Rh blood group determinations and assay of erythrocyte catalase activity (K_{cat} activity). The assay method has been described elsewhere (Takahara *et al.*, 1960).

When the first unusual K_{cat} values were obtained in the present study, the assay method was checked using fresh blood samples from several family members as well as from other previously known normo- and hypocatalasic individuals. The average variation between paired determinations was 0.11 K_{cat} units, well within the limits of error of the determination, confirming previous studies here and elsewhere that demonstrated the constancy of erythrocyte catalase activity in any one individual over considerable time intervals (Richardson *et al.*, 1953; Takahara *et al.*, 1960; Wyngaarden and Howell, 1960). Hence the K_{cat} values reported below are considered representative of the true state of erythrocyte catalase activity and not spurious values caused by technical errors or laboratory "drift."

RESULTS

Fig. 1 shows the pedigree for the entire kindred and table 1 gives the K_{cat} values and other pertinent data for each person from whom a blood sample was obtained. K_{cat} values were determined for a total of 110 related individuals in the kindred, 58 males and 52 females, and from six unrelated spouses. The individuals designated as hypocatalasic in the pedigree all had values below 3.81; the range of normal in this laboratory is 3.90-7.50 units. ABO, MN, and Rh blood groups were not unusual and conformed to the expected inheritance patterns; there were no paternity exclusions.

In Fig. 2 the K_{cat} values are arranged in order of magnitude; it is immediately apparent that these data do not correspond to the non-overlapping trimodal distribution of K_{cat} values earlier reported in the families of acatalasics. Here, in contrast, is a broad continuum (zero values aside) ranging from the lowest, 2.98, to the highest, 6.73, with a distinct peak at 5.40-5.59, corresponding to the previously established mean of 5.38 for a group of 259 randomly selected individuals. The lowest K_{cat} values in the present report (aside from those of the three acatalasics) are at the upper limit of values reported previously for

TABLE 1. THE K_{CAT} VALUES ENCOUNTERED IN THE OH KINDRED

Pedigree Position	Sex	Age	K_{cat}	Pedigree Position	Sex	Age	K_{cat}	Pedigree Position	Sex	Age	K_{cat}
V	1	92	3.65	VI	27	35	6.07	VII	9	7	6.64
	2	83	3.23		(28)	44	4.46)		10	12	6.50
	5	86	4.31		29	37	4.65		11	36	6.34
	6	82	4.18		30	40	3.09		12	20	5.51
	10	79	5.46		31	51	3.64		13	19	6.36
	13	71	4.14		32	48	5.31		14	15	5.43
	14	74	4.62		33	46	3.75		15	12	5.35
	15	63	4.53		34	44	3.47		16	24	0
	16	61	6.16		35	40	5.04		17	20	4.54
	17	78	4.64		36	49	5.30		18	18	0
	18	73	4.73		37	47	5.58		19	15	5.83
	19	65	5.41		38	45	5.45		20	13	5.92
	20	84	5.11		39	39	5.65		21	9	5.09
					40	32	5.37		22	15	5.76
					41	30	5.44		23	12	5.42
					(42)	40	5.95)		24	11	5.80
					43	43	3.10		25	11	5.51
					(44)	31	5.44)		26	11	0
					45	39	5.01		27	9	2.99
					46	38	5.61		28	4	2.98
					47	32	4.80		29	26	5.46
VI	1	60	5.15		48	29	4.93		30	24	4.45
	2	57	3.56		49	26	2.93		31	23	3.74
	3	50	4.78		50	23	3.06		32	14	5.05
	4	60	3.65		51	27	5.71		33	16	5.95
	5	55	5.14		52	56	5.74		34	14	5.50
	6	65	3.50		53	41	5.53		35	12	6.22
	7	65	3.34		54	37	6.12		36	18	6.13
	(8	55	5.15)		55	37	6.23		37	14	4.41
	9	59	4.44		56	42	4.21		38	11	4.70
	10	50	5.95						39	13	4.78
	11	53	5.86						40	11	4.56
	12	50	5.20						41	5	5.38
	13	52	5.45						42	14	5.95
	14	47	4.03						43	10	5.36
	15	58	5.42						44	15	4.52
	16	53	5.60						45	5	5.65
	17	37	5.20						47	10	5.65
	18	54	4.45	VII	1	16	5.28				
	19	51	4.35		2	29	4.45				
	20	46	5.85		3	28	5.05				
	(21	56	5.33)		4	23	5.29				
	22	47	5.85		5	20	3.80				
	24	42	5.56		6	17	4.83				

hypocatalasics (Nishimura *et al.*, 1959; Takahara *et al.*, 1960; Hamilton *et al.*, 1961). The tabulation below summarizes these comparisons:

	No.	Mean K_{cat}	Range
ABCC Clinic Controls	259	5.38	3.90-7.47
Hypocatalasia	37	2.51	1.94-2.98
Present Report	116		2.98-6.73

Three acatalasic individuals were encountered, the 18-year-old female proband (VII-18), a male sibling (VII-16), and a male cousin (VII-26). The *apparent* residual activity in these three persons ($K_{cat} = 0.002$, 0.0003, and 0.000, respectively) is so low as to be well within the error of the method, *i.e.*, there appears to be complete absence of catalase activity. None had ever experienced the oral gangrene that is reported to occur among about half the acatalasic individuals thus far recognized (Takahara, 1961). The proband's parents are first cousins; the mother is dead. The K_{cat} for the father (VI-9) is not in the range of values of the earlier reported carriers, but falls within the range for normal individuals established by previous studies (Nishimura *et al.*, 1959; Takahara *et al.*, 1960; Hamilton *et al.*, 1961). Two paternal sibs and a child of one have K_{cat} values below normal (VI-6, 7 and VII-5). The proband's paternal grandparents are third cousins. The K_{cat} of the grandmother (V-5) is in the lower normal range. Two sibs of the dead paternal grandfather (V-4) as well as an offspring of one of these sibs also have low K_{cat} values (V-1, 2 and VI-2).

The acatalasic cousin (VII-26) of the proband has two sibs with abnormally low K_{cat} values (VII-27, 28). Their parents are first cousins, and although the K_{cat} of the father (VI-30) is below normal, that of the mother (VI-29),

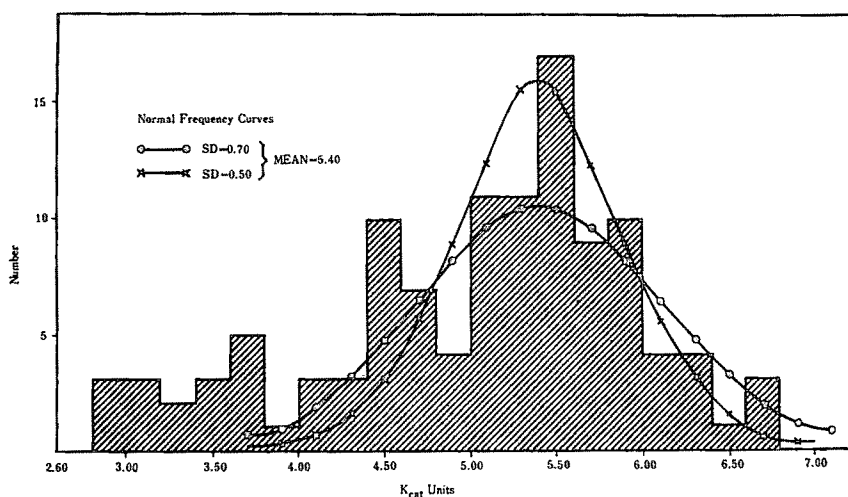


FIG. 2. Distribution of K_{cat} values. OH kindred.

an aunt of the proband, is in the lower range of established normal. The maternal grandmother (V-10) of the three acatalasics has a K_{cat} well within the normal range, being very close to the previously established normal mean of 5.38 (Hamilton *et al.*, 1961). Her husband, the maternal grandfather (V-8), who is also her third cousin, is dead; so far no unusual K_{cat} values have been found among the offspring of his sibs.

Among the paternal sibs of the proband's acatalasic cousin (VII-26) are three with low K_{cat} values, one of whose children also has a low K_{cat} (VI-31, 33, 34 and VII-31).

Further right on the pedigree are three individuals with low K_{cat} values (VI-43, 49, 50); the parents of these three are third cousins and the K_{cat} values of both are within the normal range (V-14, 15). A paternal aunt (V-13) and all her offspring so far tested have normal K_{cat} values; similarly, a search among relatives of the maternal grandfather has not revealed any abnormal K_{cat} values (*e.g.*, V-17 *et seq.*).

The pedigree shows three third cousin marriages in generation V and four first cousin marriages in generation VI. Consanguinity was also said to have occurred in generation I, but the exact relationship is unknown.

GENETIC ANALYSIS

The genetic analysis of this extended kindred presents a number of interesting problems. In all previous studies on acatalasia in Japan, the catalase values in the heterozygous parents were clearly outside the normal range. However, in this kindred there are two instances of an acatalasic child whose (heterozygous) parent has a value in the normal range. It follows that other heterozygotes in the family may also have values in the normal range, thus creating difficulties in the evaluation of the carrier state in this kindred.

Two obvious alternative approaches exist in this situation. One could assign each possible carrier a weighted probability that he is in fact a carrier, and develop a picture of the carrier state based on this weighted probability and the catalase value observed in that individual. This method will be dealt with elsewhere (Hamilton and Hashizume, in manuscript). An alternative approach, and the one we will pursue in this paper, attempts to reduce the observed distribution of catalase values in the kindred to two subdistributions. This may proceed in either of two ways. In the first, more empirical approach, we assume that the normal members of this kindred have a distribution of catalase values similar to and perhaps identical with those encountered in previous studies of normal individuals. We assume further (and will give supporting evidence later) that no heterozygote has a catalase value exceeding the normal mean. The previously observed mean and standard deviation for normal individuals was in one series of observations (Takahara *et al.*, 1960) 5.38 ± 0.73 units, and in a second series of observations (Hamilton *et al.*, 1961) 5.30 ± 0.53 units. A third set of normal values (Takahara *et al.*, 1960) yielded an estimate of 4.97 ± 0.61 units, but these were obtained after some of the specimens had been stored for several days; it was pointed out at the time that these values were very likely too low, and subsequent experience has confirmed this impression.

The present data (Fig. 2) show a sharp mode at 5.40-5.59 units, suggesting that in the normal members of this kindred the mean might be somewhat greater than 5.40. A certain measure of arbitrariness becomes necessary—we shall make two calculations, one assuming a mean of 5.40 and a standard deviation of 0.70, and the second assuming the same mean but a standard deviation of 0.50.

In the present data, 47 individuals fall above this assumed mean, all of whom we assume to be normal. If we assume a symmetrical distribution of normal values about the mean, then a similar number should fall below that value. We are thus led to expect 94 normal individuals in the sample. In Fig. 2 we have plotted the normal frequency curves for (1) a sample of 94 individuals with a mean value of 5.40 and a standard deviation of 0.70 and (2) a sample of the same size and with the same mean, but a standard deviation of 0.50. That either set of assumptions is permissible is indicated by the fact that in neither case is there a significant difference between the observed values in the descending limb of the curve and the expected values (Case 1: $\chi^2 = 5.69$, $df = 5$, $.3 < P < .5$; Case 2: $\chi^2 = 3.60$, $df = 5$, $.5 < P < .7$). An estimated distribution of catalase values in heterozygotes may be derived in both cases by assuming that the excess of observation over expectation on the ascending limb of the curve is due to the heterozygous individuals. A clear defect in this approach is that *all* entries in excess of expectation at any point on the ascending limb of the curve are assigned carrier status, whereas in fact some of this excess may be accounted for by random fluctuation, just as there are intervals in which a deficiency of entries is to be expected. With the first set of assumptions, 23 individuals are assigned carrier status; the distribution thus derived is shown in Fig. 3a. In Fig. 3b is given the similarly derived distribution on the second set of assumptions. Now we assign 29 individuals carrier status. The mean of the values in Fig. 3a is 3.70, while for those in 3b, it is 3.80. In the first case an interval of 0.30 catalase units separates the highest carrier value from the mean for normals, while in the second case the interval is 0.70. The interval is sufficient to give some confidence in the original assumption that no carrier value was higher than the normal mean. Incidentally, in Fig. 3a there is no entry in the 4.60-4.79 interval, even though a known (heterozygous) parent of an acatalasic child was found to have a value of 4.65; this presumably results from a deficiency of non-carrier entries in this interval and represents another imperfection in the approach.

A second approach to obtaining the distribution of carrier values stems from Rao's method for computing from a distribution assumed due to two overlapping normal frequency curves, the means and standard deviations of the two curves involved (Rao, 1952). This method assumes the two distributions to have the same standard deviation. Inasmuch as inspection suggests the carrier curve to be more platykurtic than the curve of normals, this assumption may not be entirely warranted. Furthermore, the total number of observations represents the bare minimum consistent with the application of this method. Be this as it may, this method yields estimates of the two means of 3.76 and 5.46, while the estimates of the population standard deviation (both populations) is 0.54.

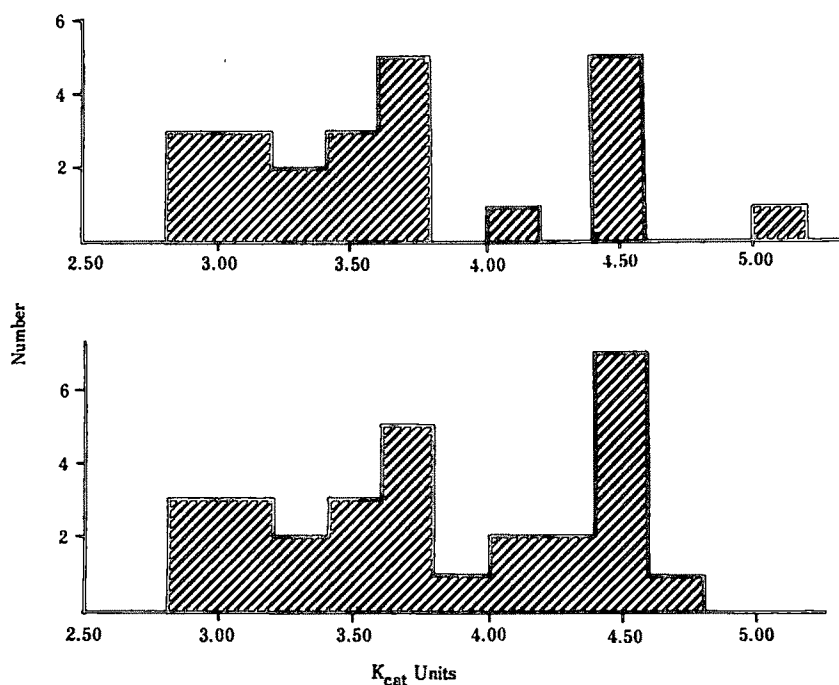


FIG. 3. Computed distribution of carrier K_{cat} values. OH kindred. a. Mean normal $\pm \sigma = 5.40 \pm 0.70$; mean carrier = 3.70; number of carriers = 23. b. Mean normal $\pm \sigma = 5.40 \pm 0.50$; mean carrier = 3.80; number of carriers = 29.

Furthermore, the proportion in the carrier category is estimated to be 0.25, or 28.1 individuals. The agreement between these figures and those resulting from the second set of assumptions in the first method pursued (which also yielded the better fit to the observed distribution) is nothing less than striking.

In our first study of the carrier state (Takahara *et al.*, 1960) a mean value of 2.17 ± 0.35 units was observed. It was pointed out that this value was probably too low, due to loss of catalase activity with storage. A subsequent study (Hamilton *et al.*, 1961), using fresher blood (corresponding to the conditions of the present observations), yielded carrier values of 2.51 ± 0.27 units. A comparison of the results of these earlier studies with the carrier values derived in the present study shows the latter to have a flatter distribution curve which exhibits no clear modal value, a higher mean value, a greater range, but no single value falling below the previously observed mean value for carriers.

Although numerous explanations of the unusual findings in this kindred can be invoked, two possibilities would appear to warrant particularly serious consideration:

Hypothesis 1

The findings are best explained by the occurrence in this kindred of two different but allelic genes for acatalasia, one the previously described gene, the other new. The existence (on this hypothesis) of a previously unrecognized

gene follows from the observed high catalase values in two proved carriers. Against the additional presence of the previously described gene is the fact that no carrier value in this kindred falls below the mean of the previously observed carrier value, whereas if, say, some eight of the calculated 23 to 29 carriers (a conservative estimate) possessed the previously recognized gene, four should have presented with values below that mean. Since, furthermore, the frequency of carriers for the previously recognized gene is by actual survey only one per 1,000 individuals in the Hiroshima area (Hamilton *et al.*, 1961), it would be most unlikely that this gene was also present. This improbability is underscored by the fact that both of the sibships in which homozygotes are found are the issue of first cousin marriages, and the mothers of these two sibships are sisters and the fathers first cousins.

Hypothesis 2

The findings are best explained by the presence of a previously undescribed gene, which may or may not be allelic to that already described, apparently somewhat more sensitive in its manifestations to modifying influences than the previously characterized mutant. We note, first, that the complex pattern of consanguineous marriage in this family provides ample pathways for all the parents of the two segregating sibships to possess the same gene. We note, further, that five of the six lowest values observed cluster in two sibships, V 43-50 and VI 26-28. While the impossibility of identifying with certainty individual carriers precludes an analysis of the variance between and within sibships, the above observations strongly suggest significant intra-sibship similarities. The postulate of one gene subject in its expression to important modifying influences thus appears quite reasonable.

The nature of these modifying influences is obscure. The "peaking" on the upper end of the distribution curve of carrier values raises the possibility of a rather sharply segregating, modifying mechanism, if the mechanism is indeed genetic. In the present state of our knowledge, there are a number of tenable hypotheses, not mutually exclusive, regarding this mechanism. Thus, one could postulate that this particular allele, as compared with the usual form of the mutant gene, is more sensitive to the action of genetic modifiers, which might be termed "partial suppressors." Or, we might attribute these variations to the effect on the expression of this particular gene of segregating isoalleles of the normal gene, comparable to the "cubitis interruptus" situation in *Drosophila* (Stern and Schaeffer, 1943). In man, a similar mechanism has been invoked to explain variations in the amount of the abnormal hemoglobins (Itano, 1953) and of pseudocholinesterase (Kalow and Staron, 1957; Kalow, 1962; Liddell, Lehmann and Silk, 1962).

Neither of these suggestions satisfactorily accounts for the higher mean values in these heterozygotes as compared to those previously studied. We might, in this connection, postulate that the regulation of catalase production normally involves feedback inhibition, which is decreased in the case of this new mutant. More specifically, we might postulate that the usual (first recognized) mutant gene results in the production of a protein which although unable to discharge

its normal enzymatic functions, can, like the normal gene, regulate by feedback inhibition the amount of enzyme-protein produced. However, in the case of this new mutant, it could be that either no catalase protein is produced or the enzymatically inactive protein which is produced exercises its regulatory qualities in a variable manner. While this is an attractive hypothesis, it appears to be comprised by the immunologic and electrophoretic evidence suggesting that in the (usual) acatalasic, catalase-type protein either may be absent or somewhat reduced in amount (Takahara *et al.*, 1962). Further speculation must await additional biochemical studies in all the types of acatalasia.

DISCUSSION

Data from the present kindred appear to establish the fact that there are at least two forms of acatalasia in Japanese. In addition, data presented in an earlier report suggest that there may be yet another variant: a single male acatalasic was found, all three of whose offspring had catalase values well within the normal range, suggesting completely recessive inheritance (Hamilton *et al.*, 1961). Complete paternity exclusion studies were not done. For these three offspring the mean value was 4.95 units, which while a little (insignificantly) below the normal mean is above the carrier values of the present paper. While on such limited data we cannot exclude the possibility that this is an extreme (normal) manifestation of the heterozygous state described in the present paper, we think it unlikely. There are thus already grounds for suspecting three genetic types of acatalasia among Japanese. The apparent variability in the manifestations of the carrier state in the Swiss kindreds segregating for a "recessive" gene resulting in acatalasia, and the overlap of carrier values with normal (Aebi *et al.*, 1962/1963), would seem to relate this gene more closely to the one described in the present communication than to the more usual type in Japan. Plainly, without detailed family investigation, the various genetic types cannot be distinguished one from another.

Because many carriers of the acatalasia variant reported here would be missed with current screening techniques, and, of course, carriers for the completely recessive type would not be detectable at all, the figures in our earlier paper concerning the frequency of the carrier state in Japan (Hamilton *et al.*, 1961) are minimal. However, the number of undetected (because undetectable) carriers is unlikely to be sufficiently great to upset the principal conclusion of that paper, namely, the very uneven distribution of carriers throughout Japan.

The methods utilized here to arrive at an approximation to the mean enzymatic values in carriers would seem to have a rather general applicability where carrier and normal values overlap and a rare trait is involved. Only a sample of normals and a group of segregating kindreds are necessary. Possibly the most important practical consideration is that all determinations be done in the same laboratory with the same technique.

Further refinements in the biochemical definition of these diseases are necessary before the differences between the variants can be characterized more precisely. The assay methods give a quantitative measurement of catalase activity in the blood but no information with respect to qualitative differences. Elec-

trophoretic studies of partially purified blood extracts from the most common type of acatalasia, with the readily identified heterozygote, have so far not demonstrated unequivocally a genetically altered protein (Takahara *et al.*, 1962), but the technique recently employed by Baur (1963) to demonstrate an atypical erythrocyte catalase has not yet been applied to this or any other of the Japanese families with acatalasia. The results of immunochemical studies do not permit one to differentiate between the total absence of the catalase protein and its alteration to the point where immunologic specificity is lost (Kajiro, 1958; Otani, 1960), nor do these studies permit a distinction between carriers and normals (Nishimura *et al.*, 1961). Methods used in such studies apparently are not sufficiently sensitive to make these distinctions but other more refined immunochemical techniques may demonstrate differences hitherto undetected (Higashi *et al.*, 1960; Hirai *et al.*, 1961). Cell cultures have been developed from skin biopsies donated by an acatalasic of the previously described variety and a hypocatalasic relative. Catalase assay of sonicates from these lines indicate that the three phenotypes are distinguishable even after the cells have grown *in vitro* for long periods of time (Krooth, Howell and Hamilton, 1962). The ability to study the defect in autonomously replicating cells under a variety of conditions adds a new dimension to the characterization of catalase abnormalities. When the above and other techniques are used to investigate further the kindred presented here, differences between this and the more common variant may be found that will help to elucidate the as yet unknown fundamental defect in acatalasia.

SUMMARY

Catalase values have been determined on the blood of 116 members of an extensive kindred in which two sibships are segregating for acatalasia. In contrast to previous studies, in which a clear distinction was observed between the values in heterozygous carriers of the gene and normal individuals, in this kindred carrier values overlapped with normal. Two methods were employed to obtain an estimate of the distribution of carrier values. The best estimate appears to be a mean of 3.70-3.80 catalase units (as contrasted to a normal value of 5.40) with a range from 2.90 up to 4.70 or, less likely, 5.10 units.

ACKNOWLEDGMENTS

We are indebted to Dr. W. J. Schull for helpful discussion regarding some of the statistical problems involved.

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1,287 Negroes and 2,090 Whites. Details of the census and study population appear elsewhere (McDonough *et al.*, 1963).

Subjects studied for genetic traits were among those seen at the Claxton Heart Research Project Center in late 1960 and early 1961. They were taken largely from the second through the sixth samples of the study population defined as above. Age, sex, race and other data were recorded for all residents, as well as the livelihood and education of heads of households, for use in calculation of an index of social status (McGuire and White, 1955). Results of the census were in close agreement, with respect to age, race and sex, with the Census Bureau enumeration of the population in the same year. Demographic characteristics of the samples studied for each trait and the total study population are given in table 1.

During the medical examination 20 ml of whole blood was drawn by venepuncture from each subject, using "Vacutainer" tubes (Becton-Dickinson Co.). About 12 ml of this blood was collected in tubes containing sterile acid-citrate Dextrose (A.C.D.) solution and the remainder in dry sterile tubes.

Hemoglobin Types

Blood collected in A.C.D. was refrigerated at 4° C within four hours. About 1 ml of red cells were washed three times in 0.84 per cent saline and stored at minus 20° C. Several months after collection, frozen red cells were thawed, diluted to approximately 10 gm per 100 ml of hemoglobin, mixed with toluene, and centrifuged. The supernatant was converted to cyanhemoglobin by standard methods.

TABLE 1. CHARACTERISTICS OF THE TOTAL STUDY POPULATION AND OF THE SAMPLES USED IN THE GENETIC STUDIES

Population and Samples	No.	% over 39 yr. old	Mean Age	% Males	% from Bullock County
Negroes					
Total Study Population	1,287	73	40.9	46	24
Hemoglobins	247	58	41.2	42	25
G6PD Deficiency	173	65	43.6	44	28
Haptoglobins	167	70	46.7	41	22
Transferrins	133	73	47.6	41	22
Red Cell Antigens	304	60	42.1	38	21
Whites					
Total Study Population	2,090	69	40.7	49	0
Hemoglobins	(Not studied)				
G6PD Deficiency	91	58	44.0	48	0
Haptoglobins	145	69	48.4	41	0
Transferrins	107	78	48.9	40	0
Red Cell Antigens	333	68	46.2	47	0

Hemoglobin specimens were submitted to electrophoresis in starch gel using the discontinuous buffer system of Poulik (1957). Specimens with patterns of hemoglobin S were also submitted to agar gel electrophoresis (Robinson *et al.*, 1957). Thirty-one specimens which appeared to have increased amounts of hemoglobin A₂ were studied with starch block electrophoresis through the courtesy of Dr. Howard Pearson, National Naval Medical Center, Bethesda, Maryland. Hemoglobin A₂ values above 3.5 per cent were considered abnormal. Six of these 31 specimens had elevated hemoglobin A₂. Hemoglobin F levels were determined using the alkali denaturation method (Singer, Chernoff and Singer, 1951); concentrations above 2 per cent were considered abnormal. Only specimens with at least 20 per cent of alkali resistant hemoglobin could be detected using the starch gel patterns.

G6PD Testing

Red cell G6PD activity was determined by dye decolorization (Motulsky, 1961) on blood collected in A.C.D. from 178 Negroes and 96 casually selected White in samples 3 to 6. Testing was done at room temperature (24° to 27° C)

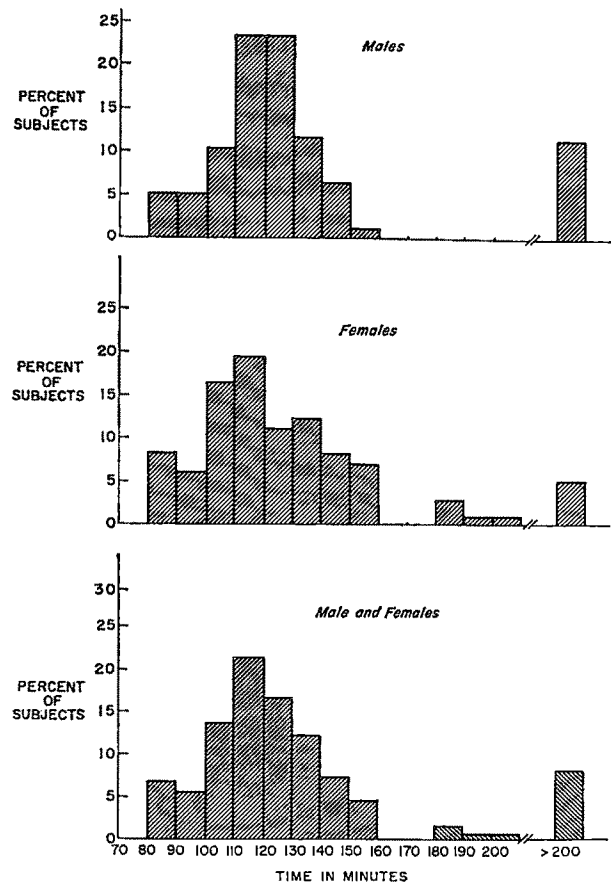


FIG. 1. Red cell G6PD decolorization times.

within 12 to 36 hours of blood collection. Most specimens decolorized before 155 minutes, and this value was taken as the normal upper limit (Fig. 1). There was no evidence that hematocrit levels (determined by the microhematocrit method) affected the decolorization time.

Haptoglobins and Transferrins

Haptoglobin and transferrin phenotypes were determined on the sera which had been stored at minus 20° C. Sera with no detectable haptoglobin were subjected to repeat electrophoresis before they were classified as type O. Haptoglobins were determined by methods previously described (Blumberg and Gentile, 1961) using the horizontal starch gel method (Smithies, 1955) with the discontinuous buffer system (Poulik, 1957). All serum specimens which were not clearly type CC transferrin were re-studied with vertical starch gel electrophoresis (Smithies and Hiller, 1959). Radioactive Fe⁵⁹ was added to these sera and radioautographs made as described elsewhere (Blumberg and Warren, 1961). Specimens of B₂C and CD₁ transferrin were included in all vertical runs as controls.

Red Cell Antigens

Red cell antigens were typed by the Blood Grouping Laboratory in Boston. In the Rh system, Cde was considered absent and C^w was assumed to be present only as C^wDe. Only D (-) cells were tested for D^u.

Other Polymorphisms

The PTC taste test was done by the sorting method (Harris and Kalmus, 1949). The gamma globulins (Gm), β -lipoprotein antigens, and the groups specific (Gc) traits are to be reported elsewhere.

RESULTS AND DISCUSSION

The hemoglobin, haptoglobin and transferrin phenotypes and gene frequencies are given in table 2. These traits were studied in subsamples of "unrelated" individuals, that is, individuals with neither parents, children nor sibs in the subsample, so that the frequencies of the alleles would not be influenced by familial associations.

The results of the hemoglobin study on Negroes are shown in table 2. White subjects were not studied. Two men were heterozygotes for *Hb^s* and " β " type thalassemia (Gerald, 1961). Six men with otherwise normal hemoglobin had elevated levels of hemoglobin A₂, but normal levels of hemoglobin F. No β_2 variants were seen. Two subjects had hemoglobin F levels of 26 and 33 per cent, and are designated "AF" in table 2. Three of 75 others had elevations of hemoglobin F between 2.0 and 4.0 per cent. There were no specimens with an increase of both hemoglobin F and hemoglobin A₂. Subjects without detectable haptoglobin are designated "0". Of the three Negroes with this condition, one had hemoglobin S-thalassemia disease, another had G6PD deficiency, and the third had normal hemoglobin but was not tested for G6PD deficiency. One of

the ahaptoglobinemic whites had normal G6PD; the other was not tested for this trait. In calculating the frequency of Hp^1 , the 2-1m and ahaptoglobinemic subjects were omitted. Thirteen of 133 sera from the Negro population were type CD₁ transferrin, and the remainder were type CC. All but four of the 107 sera from the White population were type CC. In another study, one of these was identified as a B₁₋₂B₂ heterozygote, the first such serum described (Robinson *et al.*, 1963).

Results of tests of red cell G6PD activity in 173 unrelated Negroes are summarized in table 3. No G6PD deficient individuals were found among 95 Whites of both sexes. The distribution of decolorization times for Negro men was sharply bimodal with no values between 160 and 275 minutes (Fig. 1).

TABLE 2. PHENOTYPES AND GENE FREQUENCIES OF HEMOGLOBINS, HAPTOGLOBINS AND TRANSFERRINS IN NEGROES AND WHITES

Phenotypes	No.	Negroes Frequency	No.	Whites Frequency
Hemoglobins				
AA	214	.867	Not studied	
AS	19	.077		
AC	4	.016		
AF*	2	.008		
S-Thal†	2	.008		
Elevated A ₂	6	.024		
Total	247	1.000		
Haptoglobins				
1-1	48	.287	27	.186
2-1	61	.365	64	.441
2-2	42	.252	52	.359
2-1m	13	.078	0	.000
0 ‡	3	.018	2	.014
Total	167	1.000	145	1.000
Transferrins				
CC	120	.902	103	.962
CD ₁	13	.098	2	.018
CB ₂	0	.000	1	.010
B ₁₋₂ B ₂	0	.000	1	.010
Total	133	1.000	107	1.000
Gene Frequencies				
Hb^s		.04	Not studied	
Hp^1		.52		.41
Tf^c		.95		.98
Tf^{D_1}		.05		.01
Tf^{B_2}		.00		.01

*Subjects with hemoglobin F levels above 10%, but otherwise normal were classified to carry "high F" genes.

†Heterozygotes for both hemoglobin S and thalassemia.

‡No haptoglobin detectable by starch gel electrophoresis.

TABLE 3. RED CELL G6PD DEFICIENCY IN NEGROES

Phenotype	Males	Females	Totals
Normal	67	87	154
G6PD Deficient	9 (11.8%)	10 (10.3%)	19
Totals	76	97	173

Using the results for males only, the estimated frequency of the gene for abnormal G6PD is .118.

Specimens from seven women had intermediate decolorization times; these women are probably heterozygotes. The estimate of the frequency of the gene for G6PD deficiency ($q = .118$) is taken from the frequency of the gene in males since the trait is sex-linked and only the phenotypes can be determined in females. Assuming random mating and no selection, the expected proportion of females carrying at least one gene for G6PD deficiency would be $2q(1 - q) + q^2$ or .222. Thus .222 of 97 or 22 of the females studied would be expected to carry the gene. Ten or 45 per cent of the expected number were observed. Using the same procedure, Allison (1960) noted 41 per cent of the expected number among East African women.

The results of the PTC taste test will be reported in detail elsewhere; the

TABLE 4. ABO, MNS, AND RH PHENOTYPES OF NEGROES AND WHITES

Phenotypes	Negroes		Whites	
	No.	Proportion	No.	Proportion
ABO				
O	162	.540	172	.517
B	58	.193	20	.060
A ₁	50	.167	97	.291
A ₂	24	.080	39	.117
A ₁ B	1	.003	4	.012
A ₂ B	5	.017	1	.003
Totals	300	1.000	333	1.000
MNS				
N _s	58	.190	59	.177
NS	4	.013	1	.033
NS _s	15	.049	21	.063
Ms	42	.138	34	.102
MS	6	.020	13	.039
MS _s	22	.072	39	.117
MN _s	113	.372	79	.237
MNS	9	.030	11	.033
MNS _s	31	.102	76	.228
NS ^u S ^u ¹	3	.010	0	.000
MNS ^u S ^u ¹	1	.003	0	.000
Totals	304	.999	333	.999
Rh				
ccdde ^e	² Rh: -1, -2, -3	16	46	.139
ccddE ^e	Rh: -1, -2, 3, 5	0	7	.021
ccD ^e e	Rh: 1, -2, -3	161	9 ³	.027
ccDE ^e	Rh: 1, -2, 3, 5	49	31	.093
ccDEE	Rh: 1, -2, 3, -5	4	13	.039
Ccdde ^e	Rh: -1, 2, -3, 4	3	2	.006
CcD ^e e	Rh: 1, 2, -3, 4	58	112 ⁴	.338
CcDE ^e	Rh: 1, 2, 3, 4, 5	9	44	.133
CcDEE	Rh: 1, 2, 3, 4, -5	0	1	.003
CCdde ^e	Rh: -1, 2, -3, -4	0	0	.000
CCD ^e e	Rh: 1, 2, -3, -4	4	65 ⁵	.197
CCDE ^e	Rh: 1, 2, 3, -4, 5	0	1	.003
Totals		304	331	.999

¹Since these red blood cells were S-s- they were assumed to be S^u homozygotes.

²The notation of Rosenfield *et al*, 1962, is included for convenience here and in Table 6.

³includes 3 D^u(+)

⁴includes 2 C^u(+)

⁵includes 1 C^w(+)

frequency of the taster gene (T) was .67 in 285 Negroes and .527 in 314 Whites.

The red cell phenotypes of Negroes and Whites appear in tables 4 and 5 and the corresponding gene frequencies in table 6. The gene frequencies in the total samples (table 6) were not significantly different from the frequencies in the sub-samples of unrelated individuals. With respect to the MNS group, four of 304 Negroes were both $S(-)$ and $s(-)$ and the gene frequencies were calculated on the assumption that these individuals were homozygous S^uS^u (Race and Sanger, 1958). The estimated frequency of S^u in the Claxton Negroes was .114.

In the present study the gene frequencies of 15 polymorphic traits have been determined in a randomly selected sample of Negroes and Whites living in the same community. This information, it is hoped, will be of help in interpreting the results of the extensive cardiovascular study completed in the same population. The data permit a comparison of the frequencies between the White and Negro subpopulations without the environmental bias which might be intro-

TABLE 5. PHENOTYPES OF OTHER RED BLOOD CELL TYPES IN NEGROES AND WHITES

Phenotype	Negroes			Whites		
	No. Tested	No. Positive	Proportion	No. Tested	No. Positive	Proportion
Fy ^a (+)	304	27	.089	332	221	.666
Lu ^a (+)	304	27	.089	333	23	.069
K (+)	303	3	.010	333	28	.084
Di ^a (+)	188	1	.005	(Not studied)		
Js ^a (+)	303	70	.231	324	1	.003
P (+)	304	286	.941	333	258	.775
Wr ^a (+)	304	0	.000	333	0	.000
Be ^a (+)		(Not studied)		323	0	.000
Jk(a+b-)		166	.548		92	.276
Jk(a+b+)	303	118	.389	333	173	.520
Jk(a-b+)		19	.063		68	.204
Mg(+)	303	0	.000	333	0	.000
Vw(+)	302	0	.000	333	0	.000

TABLE 6. GENE FREQUENCIES OF THE RED BLOOD CELL ANTIGENS IN NEGROES AND WHITES

Gene		Negroes	Whites	Gene		Negroes	Whites
MS		.123	.205	O		.713	.704
Ms		.347	.303	A ₁		.097	.169
MS ^u		.014	.000	A ₂		.061	.077
NS		.055	.074	B		.129	.050
Ns		.361	.418	Jk ^a		.743	.536
NS ^u		.100	.000	Fy ^a		.046	.422
Mg		.000	.000	Lu ^a		.044	.036
Vw		.000	.000	K		.005	.042
CDe+CD ^u e	R 1, 2, -3	.103	.426	Di ^a		.003	*
cde	R -1, -2, -3	.230	.358	Js ^a		.003	.002
cDE+cD ^u E	R 1, -2, 3	.108	.148	P		.757	.526
cDe+cD ^u e	R 1, -2, -3	.535	.037	Wr ^a		.000	.000
cdE	R -1, -2, 3	.000	.017	Be ^a		*	.000
Cde	R -1, 2, -3	.025	.006				
CwDe	R 1, 2, -3, 8	.000	.005				
CDE	R 1, 2, 3	.000	.003				

*Not studied.

duced by comparing populations living in different geographic areas. It has been shown that for some loci the gene frequencies in American Negroes are different from those in the West African populations from which they are thought to have been derived (Glass and Li, 1953). In a second paper in this issue, (Workman *et al.*, 1963) the gene frequencies in the two Claxton populations will be compared to the frequencies in contemporary West Africans to ascertain the relative contributions of genetic drift, gene migration, and selection in establishing observed differences in frequencies between the West African and Claxton Negro populations.

The sera obtained during this field study have been stored and will be used for the study of other serum polymorphisms in order to obtain a more complete characterization of these populations.

SUMMARY

More than 300 Negroes and 300 Whites were randomly selected in a rural southern United States community. Their blood was studied to determine the frequencies of more than 15 biochemical polymorphic traits. The traits studied were: hemoglobin variants (in Negroes), serum haptoglobins and transferrin types, G6PD deficiency, and the red cell antigens.

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Selection, Gene Migration and Polymorphic Stability in a U. S. White and Negro Population

P. L. WORKMAN, B. S. BLUMBERG AND A. J. COOPER

National Institutes of Health, Bethesda, Maryland

IN ANY POPULATION the frequencies of the alleles associated with a polymorphic locus are related to the total genetic constitution of the population and the environment in which it is situated; changes in either would result in corresponding changes in allelic frequencies. Since polymorphic traits provide an opportunity for rapid evolutionary changes (Ford, 1960), their distributions in appropriate populations can be used to study the directed (gene migration, selection, mutation) and non-directed (chance, drift) forces which have produced the recent evolutionary trends in man (Motulsky, 1960; Blumberg, 1961; Allison, 1962).

In American Negro populations, admixture with European Whites has altered the genetic constitution (Glass and Li, 1953; Stern, 1953; Glass, 1955; Roberts, 1955) and the change in their environment, by movement from Africa to North America, could have altered the adaptive values at the polymorphic loci. In this paper, frequencies of several polymorphic traits in Negroes and Whites living in the same Southern U. S. community will be compared with the frequencies of the same traits in contemporary West African Negroes in order to help evaluate the relative roles of selection, gene migration and drift in producing the present frequencies of the traits in the American Negroes. This comparison should also indicate which polymorphisms, if any, in the American Negro population are unstable; that is, traits with significantly different adaptive values in the two populations which have not reached stable frequencies, or traits which have adaptive disadvantage in the American Negroes, but are still present because of prior advantage in Africans (transient polymorphisms). The studies were conducted in Evans and Bullock Counties, Georgia. Claxton is the county seat of Evans, and the populations, for convenience, have been called the Claxton populations. A description of the populations studied and a discussion of the techniques used for identification of the phenotypes are given elsewhere in this issue. (Cooper, Blumberg, Workman and McDonough, 1963).

ANALYSIS OF THE DATA

The frequencies of the polymorphic traits studied in the Claxton populations are presented in table 1, together with estimates of the frequencies of the same traits in other Negro and White populations and in West Africans. We have assumed that the remote ancestors of the American Negroes were mainly from West Africa (see, for example, Herskovits, 1941; Fage, 1959) and that the West African frequencies obtained from recent studies closely approximate those

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in the African populations from which the American Negroes have descended. While drift and mutation have probably had little effect on the African frequencies in the period since the Negroes came to North America, it is possible that changes in the selective values of particular traits in West Africa have caused corresponding changes in the gene frequencies in that period. Any conclusions drawn from the data must be considered with these restrictions in mind.

As seen in table 1, the frequencies of the traits in the Claxton White population are, in general, quite similar to those observed in other U. S. White and English populations, the greatest differences being approximately 5 per cent. The frequencies in the Claxton Negro differ from other U. S. Negro populations by at most 4 per cent for the majority of the alleles considered. A comparison of the frequencies given in table 1 permits the assumption that genetic drift has had no appreciable effect upon the distribution of the polymorphic traits in either the Negro or the White population in Claxton. In addition, approximately 12 to 15 generations, or 350 years, have elapsed since the arrival of the first Negroes in North America (Glass and Li, 1953; Fage, 1959); and both the Negro and White populations in Evans County each contain more than 2,400 individuals (Cooper *et al.*, 1963). From theoretical considerations, for this population size and time interval, it is unlikely that drift or mutation

TABLE 1. FREQUENCIES OF ALLELES STUDIED IN THE CLAXTON POPULATIONS COMPARED TO FREQUENCIES FROM OTHER STUDIES

Allele or Segment	West African Negro	Claxton Negro	Other American Negro	Claxton White	Other U. S. and English White
R ^o (cDe)	.594 ⁴	.535	.438 ⁴	.037	.026 ⁹ —.028 ⁴
R ¹ (CDe)	.069 ⁴	.103	.158 ⁴	.426	.408 ⁶ —.420 ⁴
R ² (cDE)	.086 ⁴	.108	.109 ¹	.148	.141 ⁶ —.150 ⁴
r(cde)	.211 ¹	.230	.264 ⁴	.358	.384 ⁴ —.389 ⁶
A	.148 ⁴	.158	.141—.188 ⁸	.246	.23 —.29 ⁹
B	.151 ⁴	.129	.093—.147 ⁸	.050	.057 —.09 ⁸
O	.704 ⁴	.713	.674—.733 ⁸	.704	.66 —.70 ⁸
M	.476 ⁴	.485	.476—.532 ⁷	.508	.533 —.547 ⁷
S	.134 ⁴	.155 [†]	.160 ⁶ —.186 ⁴	.281 [†]	.327 ⁶ —.377 ⁴
Fy ^a	.0 ⁹	.046	.053 ⁶	.422	.414 ⁶ —.434 ⁷
P	.780 ⁴	.757	*	.526	.542 ⁹
Jk ^a	.783 ⁴	.743	.732 ¹	.536	.514 ⁶ —.523 ⁷
K	.009 ⁶	.005	.018 ⁷	.042	.046 ⁶ —.066 ⁷
Lu ^a	.036 ⁶	.044	*	.036	.039 ⁶
Js ^a	*	.122	.103 ¹	.002	.0 ¹
Di ^a	.0 ¹⁰	.03	.00 ¹⁴	.0	.0 ¹⁴
G6PD	.18—.21 ¹¹	.118	.11 ¹⁰	.0	.0 ¹¹
Hb ^s	.08—.14 ⁹	.043	.02—.06 ⁷	.0	.0 ⁷
Hp ¹	.60—.78 ¹¹	.520	.531 ³ —.539 ²	.41	.43 ³
Tf ^{D1}	.035—.088 ¹¹	.049	.055 ¹²	.01	.0
T ⁺	.795 ⁵	.670	.697 ⁵	.527	.455 ⁵

*No suitable estimate could be found.

†Estimated by $S = 1 - \sqrt{S(-)}$ for purposes of comparison with West African data.

¹Giblett and Chase, 1954

²Giblett and Steinberg, 1960

³Sutton *et al.*, 1959

⁴Glass, 1955

⁵Glass and Li, 1953

⁶Race and Sanger, 1958

⁷Mourant, 1954

⁸Mourant *et al.*, 1958

⁹Allison, 1956

¹⁰Beutler, 1959

¹¹Allison and Blumberg, 1962

¹²Parker and Bearn, 1961

¹³Gershowitz, 1959

¹⁴Layrisse, 1958

has had an appreciable effect on gene frequencies, (Kimura, 1956; Moran, 1962).

The unlikelihood of significant admixture between the American Negro and the American Indian population was discussed by Glass (1955). His conclusions are supported by our finding the Di^a allele, relatively common in American Indians (Layrisse, 1958), in only one of 188 Claxton Negroes.

If the assumptions discussed above are correct, then the frequency differences between the Claxton and West African Negroes can be ascribed almost totally to the effects of gene migration resulting from admixture between the American White and the American Negro population and to differences in the adaptive values of the traits in the West African and American Negro populations.

Selection and Migration

In order to evaluate the relative effects of selection and migration, estimates have been made of the total amount of gene migration from the American White into the Claxton Negro population using the method of Bernstein (1931) which assumes that the observed differences are due to migration alone. In the following calculations it is assumed that the frequencies of the traits in the Claxton Whites are representative of the frequencies in the White population which has contributed to the Negro gene pool. If q_W , q_N , and q_{Af} are the frequencies of an allele in the Claxton White, Claxton Negro and West African populations respectively, then the total amount of gene migration, m , is given by

$$m = \frac{|q_N - q_{Af}|}{|q_W - q_{Af}|}.$$

Such an estimate of gene migration, m , for a given locus or segment, is equivalent to an estimate of the admixture, or hybridization, which has occurred between two populations only if: (a) there is no assortative or preferential mating between the two populations with respect to the locus considered; (b) the gene migration is entirely from one population into the other; (c) individuals whose genotype is derived from both populations have no special bias with respect to fertility, social factors, geographic mobility, and other factors which would affect their contribution to the gene pool of the population. For example, for an organism in which the hybrids between two populations are not viable, no amount of admixture will result in gene migration. For the present study, since we shall assume only that there has been no preferential mating with respect to the traits under consideration, the relation between the estimates of gene migration and the actual amount of admixture can not be considered. In the absence of differences in the adaptive values of the traits in West Africa and in the United States the estimates of m , computed for each of the loci, should be equal. Then, an alteration of the adaptive values of the alleles resulting either from change in environment or from modification of the gene pool by admixture would result in differences between the m values calculated for the alleles. Small variation in the m values could be ascribed to sampling accidents, drift, or small inaccuracies in the estimates of the allelic frequencies in the West African population, as well as to small changes in the

adaptive values. However, significantly different estimates of m must be the result of significant differences in the adaptive values of the alleles in the two populations.

Estimates of gene migration (m) have been calculated only for those alleles where reliable West African frequencies are obtainable and where the difference between the frequencies of the alleles in the Claxton White and West African populations, $|q_w - q_{Af}|$, is sufficiently large that the sampling error of the ratio is small. We have considered only those alleles for which $|q_w - q_{Af}|$ is at least .09. Table 2 gives the alleles considered and the corresponding values of $|q_w - q_{Af}|$ and m . The alleles or chromosome segments not suitable for this kind of analysis, for one or both of the above reasons, were O , M , R^2 , K , Lu^a , Di^a , and J_s^a .

DISCUSSION

The most striking feature of the analysis is the apparent separation of the polymorphic traits into two distinct groups. In the larger group (Group I), including all the red blood cell antigens, the m values have a range from .094 (P) to .218 (B) and a mean value of .131. The other group (Group II), which includes Hp^1 (haptoglobin), Hb^s (sickle cell hemoglobin), G6PD, T (PTC-taste test) and possibly the Tf^{D1} (transferrin) alleles, has m values which range from .34 to .70, all considerably greater than those in the first group.

In order to determine which of the two groups contains alleles whose frequencies have been primarily altered by gene migration, we should consider estimates of m obtained from alleles whose frequencies would have remained approximately constant in the West African and American White populations over the past 300 years, and for which $|q_w - q_{Af}|$ is large. Since the frequencies of Rh alleles (which all fall in Group I) are considered to be quite stable over

TABLE 2. COMPUTED VALUES OF m AND $|q_w - q_{Af}|$

Allele or Segment	$ q_w - q_{Af} $	m
Group I		
R^o	.562	.113
Fy^a	.422	.109
R^1	.357	.095
P	.266	.094
Jk^a	.247	.167
r	.147	.129
S	.147	.143
B	.101	.218
A	.098	.107
Group II		
T	.268	.466
Hp^1	.19—.38	.42—.70
G6PD	.18—.21	.34—.44
Hb^s	.08—.14	.46—.69
Tf^{D1}	.074	.495*

*The West African frequency for Tf^{D1} which was used (.088) was based on only two samples. See text for discussion.

a period of several hundred years (see, for example, Mourant, 1954) we have assumed that the m values of the Group I alleles, in the Claxton Negroes, reflect primarily the effects of gene migration. The mean of the m values for R^0 ($|q_w - q_{Af}| = .562$) and R^1 ($|q_w - q_{Af}| = .357$), namely $m = .104$, can be considered the best estimate of m in the Claxton Negro population. The variation in the m values estimated for the Group I traits (*i.e.*, the red blood cell antigens) could result from either small differences in the adaptive values of the traits in West Africa and Claxton, sampling error or genetic drift. For the Group I traits the environmental selective forces appear to be similar in West Africa and in the Southern United States. This implies that they are maintained by selective forces which operate in both ecological settings. They are, however, balanced at different levels as shown by the gene frequencies for Africans and Whites in table 1, indicating probable differences between the gene pools of the given populations. Thus, barring unknown cyclic changes which could have occurred during the generations since the movement of the Africans to North America, or selective forces which have uniformly affected the m values for the Group I alleles, gene migration, resulting from admixture between the Claxton Negroes and the American Whites, appears to be the chief cause for the differences in the frequencies of these alleles in the West African and Claxton Negro populations.

For the Group II polymorphisms, gene migration alone cannot account for the m values which are all significantly larger than .104. Nor, as noted above, could either mutation or drift have significantly influenced the frequencies of these alleles. If the contemporary West African frequencies accurately reflect the population from which the Claxton Negroes are descended then these Group II traits must have significantly different adaptive values in West Africa and Claxton. Evidence from other studies supports this hypothesis. It has been suggested that heterozygotes for either Hb^s or G6PD have an adaptive advantage in a malarial environment (Allison, 1956; Motulsky, 1960). Selection against the heterozygotes for Hb^s or G6PD would lead to a rapid decrease in the frequency of the alleles. The T allele has been considered in relation to thyroid disorders (Kitchin *et al.*, 1959). There is evidence from studies on Greek populations that the Hp^1 allele may be positively correlated with the thalassemia trait (Blumberg, 1963).

The Group II polymorphisms, T , G6PD, Hb^s , and possibly Hp^1 and Tf^{p1} , have values of m ranging from .34 to .70, indicating that these polymorphisms were, and probably still are, unstable. Since the G6PD and Hb^s alleles are almost completely absent in U. S. Whites, these traits may represent transient polymorphisms, present in the U. S. Negro because of a former adaptive advantage in the West Africans.

The present data do not provide any interpretation of the nature of the adaptive factors operating on the polymorphisms included in Group II. It should be stressed that the statistical analysis can only provide correlations between environmental conditions and allelic frequencies. Any valid interpretation of the differences in adaptive values must derive from medical or biochemical studies.

The statistical analysis of the data could be extended to a consideration of either the rate of gene migration per generation (Glass and Li, 1953; Saldanha, 1957) or the adaptive values for the traits. Such analysis would, however, entail assumptions such as constant rates of migration and fixed adaptive values, which are most unlikely, and the numbers produced would be of dubious worth.

The estimation of m for the Hp^1 gene is based on estimates of West African frequencies derived from populations in which many of the sera could not be typed because of absent or low haptoglobin levels (Allison, Blumberg and ap Rees, 1958). Although some ahaptoglobinemia is due to genetic causes (Giblett and Steinberg, 1960), much of it is probably due to the environment. Furthermore, in cases which have been recorded as ahaptoglobinemia at one time, but typable at another, the serum is often type 2-2 (Blumberg and Gentile, 1961). Hence the West African surveys may over-estimate the Hp^1 frequency. For example, if in the West African population sampled by Blumberg and Gentile (1961) half of the sera classified as type O were in fact type 2-2, the value of $|q_w - q_{Ar}|$ would be too small to permit an accurate calculation of m . Furthermore, it is now known that there are at least three alleles commonly segregating at the Hp locus (Hp^{1F} , Hp^{1S} , Hp^2) (Smithies, Connell and Dixon, 1962) and the frequency of Hp^1 is actually the sum of Hp^{1F} and Hp^{1S} frequencies.

The calculation of m for the Tf^{D1} allele is based on only a small number of West African studies. Recently several slow moving transferrin variants determined by alleles other than Tf^{D1} have been reported and in some cases the transferrin phenotypes may have been misclassified in the earlier studies. Hence the Tf^{D1} frequencies reported may be high. Furthermore, it is not unlikely that the slow moving variants reported in non-Africans are determined by different alleles. The m value calculated for Tf^{D1} must be considered tentative.

Values of m greater than .20 have been reported by Glass (1955) and Roberts (1955) from comparisons between other American Negroes and Whites. Unfortunately, they compared the frequencies of polymorphisms in Negroes and Whites who did not live in the same community and used different populations to compare different alleles. Hence, from their studies it is impossible to analyze the variation in the m values which could reflect variation in the populations considered, different amounts of admixture, or in the influence of social factors as well as adaptive differences, drift, and so forth. The frequencies of the R^0 and R^1 alleles in the Claxton Negroes are closer to the West African frequencies than are those reported by Glass (1955); Pollitzer (1958), in a study of Negroes from Charleston, South Carolina, found frequencies almost equal to those in West Africa. This variation suggests that there may be significant differences in both the amount of admixture and the amount of gene migration in different U. S. Negro populations. That is, the high estimates of m obtained from studies on Negroes living in large Northern cities could reflect either different rates of admixture or a similar rate of admixture but differential rate of geographic movement of Negroes with a high proportion of white ancestry. In Pollitzer's (1958) study, both the gene frequencies and his anthropological studies on the relative isolation of the populations suggested a low rate of admixture.

The simultaneous analysis of the distribution of several polymorphic traits has served to isolate four (or five, including the Tf^{D1}) alleles whose frequencies are, or have been, significantly altered by selective pressures which are different in the West African and Claxton populations. Additional statistical and biological studies are required to determine the nature and amount of the adaptive differences of these alleles. Similar studies in other populations should reveal additional traits which are undergoing rapid evolutionary change. The same populations, and in fact the same blood samples, may be used to determine if newly-discovered polymorphisms are balanced (Group I) or unstable (Group II). Such studies are being undertaken with the serum Gm (gamma globulin), Gc (group specific) and beta lipoprotein groups.

The variation within the group least affected by adaptive differences (Group I) should be further analyzed not only in other American Negro populations, but in populations throughout the world. In this way, the loci most stable over many generations and in different populations could be determined and used for anthropological or historical studies in these populations.

SUMMARY

The frequencies of more than 15 polymorphic traits were studied in an American Negro and White population living in the same rural Southern U. S. community and compared with the frequencies of the same traits in West African Negroes and other American Negro and White populations. It is suggested that neither genetic drift nor mutation were likely causes of the variability observed. By estimation of the total amount of gene migration, m , from the Whites to the Negroes, (under the assumption of no selection) the polymorphic traits can be separated into two distinct groups. In Group I, the larger group, which contains the red blood cell antigens, the estimates of m (.1 to .2) are consistent with the hypothesis that migration alone can account for the differences in gene frequencies between the West African and the American Negro populations. The best estimate of m was found to be .104. In Group II, containing the G6PD, Hb^s , and T alleles (and possibly Hp^1 and Tf^{D1}) the significantly higher estimates of gene migration (.4 to .7) were concluded to result from both gene migration and different adaptive values of the traits in the West African and American environments.

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Further Evidence for Linkage Between the β and δ Loci Governing Human Hemoglobin and the Population Dynamics of Linked Genes

SAMUEL H. BOYER¹
DONALD L. RUCKNAGEL²
DAVID J. WEATHERALL³
AND
E. J. WATSON-WILLIAMS⁴

¹*The Division of Medical Genetics of the Department of Medicine, The Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland*

²*Department of Human Genetics, The University of Michigan Medical School, Ann Arbor, Michigan*

³*The Division of Hematology of the Department of Medicine, The Johns Hopkins University School of Medicine and Hospital*

⁴*Department of Pathology, University College, Ibadan, Nigeria. Present address: Department of Hematology, Manchester Royal Infirmary, Manchester, England*

WITH EACH PASSING YEAR the clinical and biochemical genetics of the human hemoglobins have become increasingly complex. Four distinctly different genetically determined polypeptide chains, termed α , β , γ and δ , have been characterized to varying degrees. In addition, complex interactions between thalassemia and structural mutants (summarized by Rucknagel and Neel, 1961) and quantitative variations in the minor components have resulted in the emerging picture of diverse structural loci as well as the possible existence of quantitative regulatory loci (Neel, 1961; Motulsky, 1962). All of these loci are presumably capable of independent mutation.

The amino acid composition and sequence of the β - and δ -chains of human hemoglobin (Hb) differ at approximately eight points (Ingram and Stretton, 1961; Baglioni, 1962). Such similarity and the absence of a detectable Hb A₂, i.e., $\alpha_2^A\delta_2^{A_2}$, in all but certain primates (Kunkel *et al.*, 1957), led Ingram (1961) to suggest that the genetic structural locus governing δ -chain synthesis arose, comparatively recently in evolutionary time, as a duplication of the structural locus governing the more ancient β -chain. The differences between Hb_β and Hb_δ loci presumably developed by subsequent mutations. Gene duplication can arise by a number of mechanisms, most of which embody the process of unequal crossing over. As a result of this process the original and duplicated genes are often initially contiguous. Consequently, if duplication of the Hb_β locus has occurred, and is in fact comparatively recent, then close linkage between Hb_β and Hb_δ loci can be anticipated.

Until recently evidence for linkage between Hb_β and Hb_δ loci rested entirely with the single family observed by Kunkel, Ceppellini, Dunn and Firsheim (Ceppellini, 1959). The father of this family possessed major hemoglobins A

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and S, and minor hemoglobins, A₂ and B₂ (also referred to as A₂'). He was thus heterozygous at both *Hb_β* and *Hb_δ* loci where genes *Hb_β^S* and *Hb_δ^{B₂}* appeared to be in repulsion. Among six children there was no recombination between the two loci. An additional informative offspring was provided by a mating of a carrier of hemoglobin B₂ with a hemoglobin S and B₂ double heterozygote (Horton *et al.*, 1961). The offspring had sickle cell trait.

In 1961 Huisman, Punt and Schaad described a sizable kindred, family K, in which the gene for thalassemia and the gene *Hb_δ^{B₂}* were uniformly in coupling despite 13 certain opportunities for recombination. The form of thalassemia in family K was presumed to be determined by a gene closely linked to if not actually part of the *Hb_β* locus.

More recently Ranney *et al.* (1963) observed a family in which the genes for Hb S and for a δ-chain variant, hemoglobin Flatbush, were in repulsion. There were no recombinants among eight informative offspring, excluding the probanda. Three additional offspring (III-15, 16, 17) of a doubly heterozygous female are omitted from present consideration because two relevant consorts were not examined. Since there is a reasonably high probability that the unexamined spouse will have either of the two mutant genes, (Horton *et al.*, 1961) inclusion of such offspring would bias the following estimates of the recombination frequency in favor of a smaller value.

In the aggregate these four kindreds provide support for the existence of close linkage between *Hb_β* and *Hb_δ* loci. Nonetheless, lack of recombination in 28 opportunities is still compatible with recombination values up to approximately 0.13 at the 95 per cent confidence limit of a Poissonian distribution (Pearson and Hartley, 1958). The inclusion of Huisman's kindred K in this tally necessarily assumes a known relationship between the position of the β locus and the locus for a specific form of thalassemia. In fact, this relationship is uncertain. Thus, in a rigorous treatment of β-δ linkage, we shall limit consideration of published reports to the remaining three families. In these three families lack of recombination among 15 opportunities is compatible with recombination values up to approximately 0.25 at the 95 per cent confidence limit of a Poissonian distribution (Pearson and Hartley, 1958).

It is the purpose of the present report to provide further family evidence for β-δ linkage, to present a means whereby very close linkage can potentially be inferred from the use of population data, and to explore the population dynamics of these linked loci.

METHODS AND MATERIALS

Hemolysates containing approximately 10 gm/100 cc hemoglobin were prepared. All samples were examined by conventional starch gel electrophoresis (Smithies, 1959) or by utilizing a discontinuous buffer system at pH 8.5 (Poulík, 1957). Under such conditions major hemoglobin types, *e.g.*, A, S, and C, and minor hemoglobin types, *e.g.*, A₂ and B₂, can be easily discerned. Hemolysates from 681 unrelated Maryland Negroes became available for electrophoretic analysis as a by-product of another investigation (Boyer, Porter and

Weillbacher, 1962). These subjects were chiefly male prisoners, male female hospital personnel, or pre- and post-partum women. Analysis of lysates from Nigerians was similarly derived in great part from an unpublished study (Porter *et al.*, 1963). The majority of samples from American subjects with sickle cell hemoglobin trait were obtained from other sources. These included clotted blood originally sent to the Maryland State Health Department for serological tests for syphilis and subsequently examined by census (D.J.W.) for *in vitro* sickling. Approximately 75 specimens containing hemoglobins A and S were obtained in this manner. The remainder of samples with hemoglobins A-S were provided by hematology sections at Baltimore Hospital and the Johns Hopkins Hospital where initial detection had been through paper electrophoresis. The 57 University of Michigan samples were obtained from the parents of entire family units ascertained in conjunction with other studies or from unrelated patients ascertained through the University Hospital. Nigerians were members of the Yoruba tribe, many of whom served as blood bank donors at University College Hospital, Ibadan.

RESULTS

Hemoglobin Types Observed in a Population Survey

The number and proportions of major and minor hemoglobin types appearing among 681 presumably unrelated Maryland Negroes are shown in table 1. Among 625 subjects homozygous for the Hb_{β}^A gene, 14 had both hemoglobins A_2 and B_2 and were therefore heterozygous at the Hb_{δ} locus. Forty-four subjects had sickle cell trait. None of these sickle trait subjects had hemoglobin F. In addition, 425 samples of sickle cell trait blood from Maryland and 57 samples of unrelated Negroes in Michigan were available, giving a total of 526 samples with A-S hemoglobin. The association of β - and δ -chain types is shown in

TABLE 1. HEMOGLOBIN PHENOTYPES AMONG PRESUMABLY UNRELATED NEGROES RANDOMLY ASCERTAINED MARYLAND NEGROES

Hb Type	No.	Proportion
AA_2	611	0.8974
ASA_2	44	0.0646
AC	11	0.0162
AA_2B_2	14	0.0209
ACB_2	1	0.0015
Total	681	1.0000

TABLE 2. ASSOCIATION OF Hb_{β} AND Hb_{δ} LOCUS TYPES IN PRESUMABLY UNRELATED NEGROES

Hb_{β} Locus Type	Hb_{δ} Locus Type	
	A_2/A_2 524	A_2/B_2 2
S/A	(0.9962) 611	(0.0038) 14
A/A	(0.9776)	(0.0224)

2. Among the 526 subjects with genotype $Hb_{\beta}^A/Hb_{\beta}^S$, two were heterozygous at the Hb_{δ} locus. One individual with ASA_2B_2 exhibited *in vitro* sickling but was clinically well. The identity of S in this subject was further corroborated by mobility on agar electrophoresis (Marder and Conley, 1959). Examination of his family indicated that the Hb_{β}^S gene derived from one parent and the Hb_{δ}^{B2} gene from the other parent. The other individual was hospitalized at the University of Michigan Hospital for painless hematuria, but was otherwise healthy. His father had hemoglobins A, A_2 , and B_2 . His mother had sickle cell-thalassemia disease manifested by approximately 80 per cent hemoglobin S, 16 per cent hemoglobin A, and 4 per cent fetal hemoglobin. Three sibs had hemoglobins A, S, A_2 , and B_2 ; one had only A, S, and A_2 . In this propositus the Hb_{β}^S and Hb_{δ}^{B2} genes were likewise in repulsion.

Among 76 presumably unrelated, healthy members of the Nigerian Yoruba tribe who were homozygous for hemoglobin A, there were three with phenotype AA_2B_2 , while among 58 randomly selected, unrelated AS subjects there was one with phenotype ASA_2B_2 . This individual had a father with hemoglobins AA_2B_2 and a mother with hemoglobins ASA_2 .

Family Studies Indicating β and δ Linkage

Examination of hemoglobin types from six children of the individual with hemoglobins A, C, and B_2 ascertained at Johns Hopkins Hospital is shown in Fig. 1c. The results are compatible with a lack of recombination between the genes Hb_{β}^C and Hb_{δ}^{B2} which are thus presumably in repulsion. Since C and A_2 have similar mobilities, it was not possible to observe Hb A_2 in the proposita. However, the presence of a child with hemoglobins A and C but no B_2 indicates

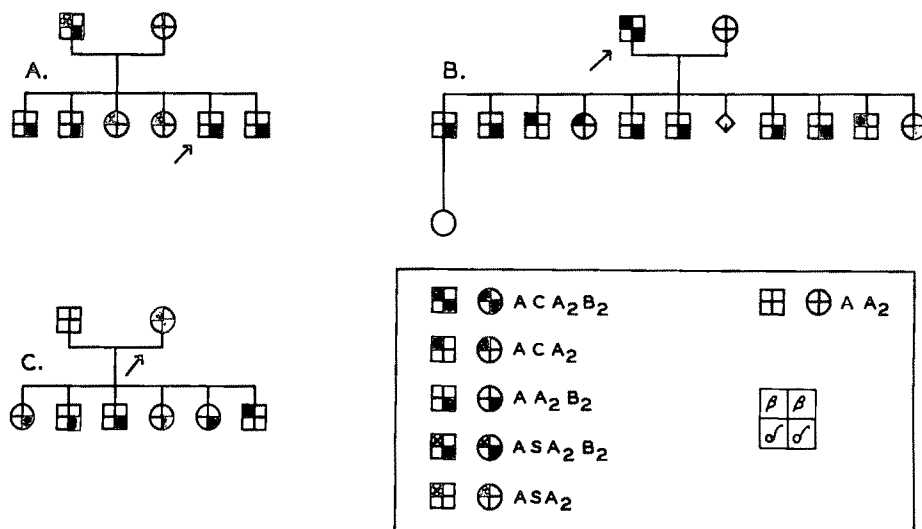


FIG. 1. Pedigrees showing segregation of genes for Hb C or S and Hb B_2 . Arrow indicates propositus (a), squares designate males, circles females.

that the proposita is probably heterozygous at the Hb_{δ} locus and possesses hemoglobin A_2 as well as B_2 .

An individual with hemoglobins A, C, and B_2 was ascertained at the University of Michigan Hospital because of severe anemia and hematologic finding compatible with agnogenic myeloid metaplasia. He has subsequently developed polycythemia vera without apparent alteration of the relative amounts of abnormal hemoglobins. His wife's blood had a normal hemoglobin electrophoretic pattern. Of his ten children, three had hemoglobins A, C, and presumably A_2 , and seven had hemoglobins A, A_2 , and B_2 (Fig. 1b). The three children without Hb B_2 indicate that the father was heterozygous for $Hb_{\delta}^{B_2}$.

Among Yoruba subjects two families with hemoglobins ASA_2B_2 were detected. One of these families, YOR-I shown in Fig. 1a, was ascertained through a proband who had hemoglobins AA_2B_2 . There were no recombinants among the five other children of the proband's father and the genes Hb_{β}^S and $Hb_{\delta}^{B_2}$ are presumably in repulsion.

In the aggregate, we observed no recombination between Hb_{β} and Hb_{δ} loci despite a total of 21 definite opportunities. The propositus of family YOR-I is omitted from the sum of opportunities.

DISCUSSION

The possibility of a sequence of loci each identifiable by precise determinations of structure at the molecular level raises questions as to the order of magnitude of recombination anticipated between these loci. The proximate limit of linkage which can exist between genes of the Hb_{β} and Hb_{δ} loci depends upon the length of the genetic unit governing the structure of a hemoglobin polypeptide chain. A rough limit for the length of such a unit may be provided by comparable data derived from study of *E. coli*. For example, the A polypeptide chain of tryptophan synthetase from *E. coli* has a molecular weight of 30,000 and its gene a maximum map distance of 2.5 units (Helinski and Yanofsky, 1962). Such observations suggest a map unit distance: chain molecular weight ratio of approximately 10^{-4} . Application of this crude estimate to a 16,000 molecular weight chain of approximately 140 amino acid residues in human hemoglobin provides a map distance of 1.6 map units. The amino acid substitution in the β -chain of hemoglobin S is at the sixth residue from the N terminus, whereas it can be deduced from the preliminary fingerprint of hemoglobin B_2 (Horton *et al.*, 1961) that an arginine molecule is substituted for glycine at the sixteenth residue from the free amino end of the δ -chain. Therefore, the distance between the two mutons should be equivalent to approximately 135 amino acid residues, or roughly 1.5 map units if, in fact, the C terminus of $Hb_{\delta}^{B_2}$ and the N terminus of Hb_{β}^S are juxtaposed. Numbers derived in this way are naturally subject to considerable reservation since there is no assurance that the physical distance of a map unit is the same in bacteria and man. To date a clear relationship between cistrons in microorganisms and pseudoalleles in *Drosophila* has not been established (Carlson, 1959). Indeed the fact that the amount of deoxyribonucleic acid per map unit is almost a thousand times larger in mammals than it is in *E. coli* (Sager and Ryan, 1961) suggests that the

10^{-4} ratio may, in man, be too large by at least several orders of magnitude, in which case the length of the hemoglobin structural loci might be expected to be of the order of 0.001 map units.

Non-genetic evidence in support of close β - δ linkage has been derived from the study of hemoglobin Lepore. Homozygotes for this hemoglobin exhibit a variant of thalassemia and lack of hemoglobins A and A₂. The elegant studies of Baglioni (1962) indicate that the non- α -chain of Hb Lepore possesses amino acid sequences at the free amino end which are characteristic of the δ -polypeptide while sequences at the free carboxyl end are characteristic of the β -polypeptide chain. Since the total number of peptides of the fingerprint add up to a chain of normal length, it seems most likely that the non- α -chain of Hb Lepore is the product of an unequal crossover between contiguous Hb_{δ} and Hb_{β} genes which lie with the C terminus of the former adjacent to the N terminus of the latter. This does not prove juxtaposition, however, and should these two loci prove *not* to be juxtaposed, then duplication either may have been more extensive and possibly involved additional but undetected loci or, alternatively, the duplication may have been translocated. Decision between contiguity and more distant linkage is desirable.

When our observations are pooled with those previously cited, there are in six families no recombinants between Hb_{β} and Hb_{δ} loci despite 36 opportunities. Close linkage between the two loci is thus indubitably established. However, these observations are still compatible, in a Poissonian distribution, with recombination values as great as 0.10, or ten map units, at the 95 per cent confidence limit and 0.148 at the 99 per cent confidence limit. If recombination is estimated by means of the binomial expansion, the values are 0.08 and 0.12 at the 0.05 and 0.01 probability levels, respectively. To reduce the upper limit of recombination to, say, two map units it will be necessary to detect no individuals having neither or both abnormalities among approximately 150 additional offspring. To establish recombination at the level of 0.2 map units, approximately 1800 such offspring would be required. Thus, an alternate approach might be desirable.

The apparent repulsion between the genes Hb_{β}^S and $Hb_{\delta}^{B_2}$ provides a potential, albeit inefficient, approach toward estimation of close linkage. Such repulsion suggests, in the absence of selection, that the mutations productive of Hb S and Hb B₂ initially arose on different although homologous chromosomes. There thus developed a population with chromosomes $Hb_{\beta}^A Hb_{\delta}^{A_2}$, $Hb_{\beta}^S Hb_{\delta}^{A_2}$, and $Hb_{\beta}^A Hb_{\delta}^{B_2}$. The appearance of the $Hb_{\beta}^S Hb_{\delta}^{B_2}$ chromosome would depend upon recombination or the less likely event of recurrent mutation. Deviation from equilibrium between the repulsion genotype ($Hb_{\beta}^S Hb_{\delta}^{A_2} / Hb_{\beta}^A Hb_{\delta}^{B_2}$) and the coupling genotype ($Hb_{\beta}^S Hb_{\delta}^{B_2} / Hb_{\beta}^A Hb_{\delta}^{A_2}$) will depend principally upon the frequency of recombination, x , between Hb_{β}^S and $Hb_{\delta}^{B_2}$ genes and upon the number of generations, N , during which there has been opportunity for such recombination. This argument assumes that there has been no selection for or against the $Hb_{\beta}^S Hb_{\delta}^{B_2} / Hb_{\beta}^A Hb_{\delta}^{A_2}$ genotype relative to the repulsion genotype. If N can be fixed at some minimum value, then a crude estimate of x can be realized by application of an expression for approach to equilibrium (Rife,

1954). The fraction of equilibrium, F , attained after N generations is related to χ by the expression

$$(1 - F) = (1 - \chi)^N. \quad (1)$$

The term F can be taken as equivalent to the expression

$$F = C_i/C_e \quad (2)$$

where C_i = frequency of chromosome $Hb_\beta^s Hb_\delta^{B_2}$ at some non-equilibrium state, e.g., at the present time, and C_e = frequency of this chromosome at equilibrium among individuals with sickle cell trait.

Estimates of the equilibrium value, C_e , are dependent upon estimates of the $Hb_\delta^{B_2}$ gene frequency. The A_2B_2 phenotype was observed in 15 of 681 unrelated randomly chosen Maryland Negro subjects. The $Hb_\delta^{B_2}$ gene frequency is, therefore, 0.01101. Similar findings have been made by Minnich *et al.* (1960), who observed Hb B_2 in 7 of 507, and Horton *et al.* (1961) in 7 of 300 hospitalized American Negroes. If these latter populations are assumed to approximate a random sample and the subjects with hemoglobin B_2 assumed to be $Hb_\delta^{A_2}/Hb_\delta^{B_2}$ heterozygotes, then a pool of these values with our own provides a $Hb_\delta^{B_2}$ gene frequency of 0.0098. Thus approximately 1.96 per cent of American Negroes should be $Hb_\delta^{B_2}/Hb_\delta^{A_2}$ heterozygotes. At equilibrium, half of all subjects with hemoglobins ASA_2B_2 should have the variant genes in coupling and half in repulsion. The value for C_e thereby derived is 0.0098. Therefore, among a group of 526 subjects with sickle cell trait there should exist, at equilibrium, 5.15 subjects with chromosome $Hb_\beta^s Hb_\delta^{B_2}$. In fact, no such subjects were observed. Although the observed value is thereby zero, the upper 95 per cent confidence limit is 3.69. Consequently, for the purpose of demonstrating *close* linkage, the value $3.69/526 = 0.00702$ is the *least* favorable value assignable to the term C_i . The use of this value will provide a test, at the 95 per cent limit, of the present method.

A minimum estimate of the number of generations, N , during which there has been opportunity for recombination between Hb_β^s and $Hb_\delta^{B_2}$ can be obtained from the study of several populations. Both genes are essentially absent among Americans of European ancestry but occur among American Negroes and the Nigerian Yoruba. Thus opportunity for recombination presumably antedates the effective close of slavery in the 18th century. The term N can thus be assigned a *minimum* value of ten generations (Glass and Li, 1953).

Where $C_i = 0.00702$, $C_e = 0.0098$, and $N = 10$, then, by equation (2), $F = 0.72$, and by equation (1), $(1-F) = 0.28 = (1 - 0.12)^{10}$. The maximum recombination value thus realized from a least favorable assumption is $\chi = 0.12$. This value approximates the maximum value $\chi = 0.10$ ($p = 0.05$) obtained from family data. However, additional data—applicable in the indirect method—probably exist in the files of several laboratories. If, for example, a total of 2,000 subjects with sickle trait failed to exhibit the chromosome $Hb_\beta^s Hb_\delta^{B_2}$, then the maximum value for C_i , at the 95 per cent confidence limit, becomes $3.69/2000 = 0.00184$ and the maximum value for $\chi = 0.02$. Among

ADDENDUM

Since preparation of this manuscript the authors have become aware of an additional family reported by Horton and Huisman (this issue, p. 394, 1963), in which three children with sickle cell trait and two with hemoglobins A, A₂, and B₂ were produced from a doubly heterozygous mother and a father with hemoglobins A and A₂. These five offspring, when added to those included herein, result in no recombinants in 41 opportunities, compatible with maximum recombination frequencies of 0.09 and 0.128 at the 95 per cent and 99 per cent confidence limits of a Poissonian distribution and 0.07 and 0.106 at the 0.05 and 0.01 probability levels of the binomial distribution, respectively.

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Risk of Fetal Death to Mothers of Different ABO and Rh Blood Types

HOWARD B. NEWCOMBE

Biology Branch, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada

ALTHOUGH THE MECHANISMS underlying selection against major blood group alleles at the ABO and Rh loci through maternal-fetal incompatibility and consequent fetal loss are understood qualitatively, information about the strengths of the selective forces is still limited.

Evidence of a possible threefold increase in the risk of stillbirth to ABO incompatible parents, for example, is based on the complete reproductive histories of 161 couples. Since only 13 stillbirths occurred, the observation is of borderline statistical significance (Reed and Kelly, 1958). Indications of a reduced fertility of ABO incompatible matings (Chung and Morton, 1961), although derived from larger numbers of parent-offspring groupings, is only indirectly applicable to estimations of mortality among the products of conception.

Still less is known about the strengths and directions of effects upon fetal mortality of various combinations of alleles at the ABO and Rh loci, although ABO incompatibility is believed to protect to some degree against sensitization of Rh-negative mothers by Rh-positive fetuses (Levine, 1958; Cohen, 1960). An apparent net increase in fertility among ABO incompatible couples has been reported from a study of 558 partial family groupings, the members of which had been ascertained in the course of a mass blood typing operation (Reed and Ahronheim, 1959). However, no quantitative measurements have yet been made of the effects on fetal mortality of simultaneous incompatibilities at both loci that would support or reject the assumption of a net favourable influence of ABO incompatibility on fetal survival arising out of interactions with the Rh system.

Some of the selective effects may be slight and may differ under varying circumstances, so that large quantities of data will perhaps be required for definitive studies of the various interactions. Precision in certain kinds of comparisons may thus be possible only when some systematic attempt has been made to interrelate the extensive blood group information currently being gathered with accurate family reproductive histories of the same individuals as set down in other records such as those of the vital statistics systems. In the meantime, however, further fragmentary evidence from additional sources can add substantially to what is now known.

The opportunity to obtain strictly relevant information on a rather large scale

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was provided by records of all reported fetal deaths occurring in the City of New York over the years 1954 through 1959, plus a ten per cent sample of all reported live births accumulated at Indiana University by Professor T. M. Sonneborn for the purpose of studying effects of paternal age on fetal loss. These records yielded 27,260 fetal deaths and 53,100 live births for which the maternal ABO and Rh blood types had both been recorded. The mother's age at the time of the birth was also available and was used in some of the analyses. Data of the kinds provided by these records allow comparisons to be made of the risks of fetal death to mothers who are capable, or incapable, of having fetuses incompatible with themselves because of ABO or Rh differences, singly or in combination.

MATERIALS

A total of 127,987 New York City fetal death cards representing all registrations of such events in the years 1954 through 1959, plus 101,013 cards for live births representing a systematic ten per cent sample, were read onto magnetic tape by an IBM 1401 computer, and tabulations were carried out by an IBM 709 computer using a generalized "101 Simulator Program" developed by the Indiana University Research Computing Center. The work was done at the Research Computing Center as part of a long-term study of paternal age effects initiated by Professor T. M. Sonneborn (1956).

The variables represented simultaneously in the tabulations used for the present analysis were ABO and Rh blood types of the mother, together with maternal age, separately for fetal deaths and for live births. No attempt was made to exhaust the relevant information content of the original punchcard records and the magnetic tape images of these cards. Still available for future study are details of sex, birth order, legitimacy, plurality, color, previous stillbirths and child deaths, gestation period, birthweight, and the delivery including operative procedures, maceration of the fetus, autopsy, and cause of death. Rh blood type of father is also recorded in a small proportion of cases, perhaps for 2 to 3 per cent of all live births and fetal deaths as indicated by a small sample.

Except where saved for specific scientific purposes, all such information, in its machine readable form, has in the past been systematically destroyed because of the difficulty of storing large quantities of punchcard records, a common practice in vital statistics systems. Information from current punchcards for births in New York City may perhaps be retained in more compact form as magnetic tape images. Unfortunately, however, blood types of parents have not been punched since 1959, presumably because of the limited use to which the information has been put.

New York City fetal death records are unique in that all products of conception are registerable by law, regardless of gestation period. Of the fetal deaths registered, approximately 30 per cent (27,270 of 92,075 for 1954-59) occurred in the period 0 to 9 weeks. Failures of mothers to be blood typed early in their pregnancies may considerably reduce the proportion of such early losses represented in the present comparisons. Nevertheless, limited information on

the risk of loss to mothers of various blood types at different stages of pregnancy might perhaps be derived from the present files in quantities adequate for future investigation of the timings of selective fetal eliminations.

One of the purposes of the present account is to indicate the extent to which existing sources of genetic information, especially those relating to selection, are currently underutilized even where the facts are already in a form suitable for certain limited kinds of investigation.

STATISTICAL METHODS

The procedures used (a) to calculate the relative incidence of fetal death for one maternal blood type as compared with another, (b) for combining such values derived from different ages of mother into a single weighted mean relative incidence, and (c) for carrying out chi-square tests of significance of the deviations from unity of relative incidence and of mean relative incidence, are those developed by Woolf (1955) and described in detail by Roberts (1957). These methods were applied by the above authors to studies of associations of blood groups with susceptibility to disease, and have been adapted to their present use.

ABO AND RH DIFFERENCES, CONSIDERED SEPARATELY

As might be expected, mothers of blood type O, in whom fetuses of types A, B, or AB would be regarded as potentially at risk from ABO incompatibility, have more fetal losses than do mothers of non-O blood types (table 1). The effect is substantial, being equivalent to a 7 per cent increase, and is statistically significant. AB mothers have the fewest fetal deaths while those of types A and B fall between the two extremes as would be predicted on simple theory. The risk to AB, A and O mothers, in fact, varies linearly with the predicted numbers of incompatible fetuses (Fig. 1).

Unexpectedly, however, the relative risk to mothers of type B is much less than would be estimated. The effect is too large to be due to differences in the frequency of the B allele in different social or racial groups; when the predicted

TABLE 1. MATERNAL ABO BLOOD TYPE AND RISK OF FETAL DEATH

Blood Type of mother	Fetal Deaths	Live Births (10%)	% Fetal Deaths*	Relative Frequency	(d.f. $\chi^2 = 1$)
O	13,454	25,341	5.31		
B	3,614	7,216	5.01		
A	9,133	18,394	4.97		
AB	1,059	2,149	4.92		
Combined	27,260	53,100	5.13		
<i>Comparisons</i>					
O/non-O			5.31/4.98 =	1.07	17.3
O/AB			5.31/4.92 =	1.08	3.9
B/AB			5.01/4.92 =	1.02	.2
A/AB			4.97/4.92 =	1.01	.1

* Among mothers who have been blood typed.

numbers of incompatible fetuses are based on allele frequencies for Negro populations, in which B is more common, the seeming anomaly remains.

The relative expectations of loss from ABO incompatibilities will differ from those shown in Fig. 1 if an interaction is assumed between the ABO and Rh loci such that Rh incompatibility protects against death due to ABO incompatibility. However, when only the single ABO incompatibilities are considered (ignoring cases of combined ABO-Rh incompatibility) the lower-than-expected risk to mothers of type B remains (Fig. 2).

Rh-negative mothers, like those of blood type O, are more prone to fetal deaths than are their antigen positive counterparts (table 2). However, the over all difference in the case of the Rh blood types is surprisingly small,

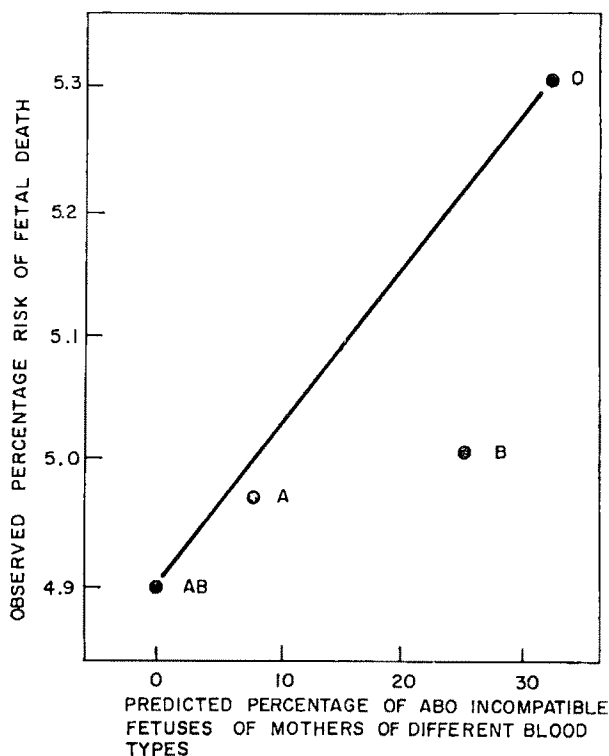


FIG. 1. Observed fetal death risk and predicted ABO incompatibility frequency for mothers of different ABO blood types.

TABLE 2. MATERNAL Rh BLOOD TYPE RISK OF FETAL DEATH

Blood Type of Mother	Fetal Deaths	Live Births (10%)	% Fetal Deaths	Relative Frequency	χ^2
Rh-neg	4,594	8,786	5.23		
Rh-pos	22,666	44,314	5.12		
Comparison Rh-neg/Rh-pos			$5.23/5.12 =$	1.02	1.1

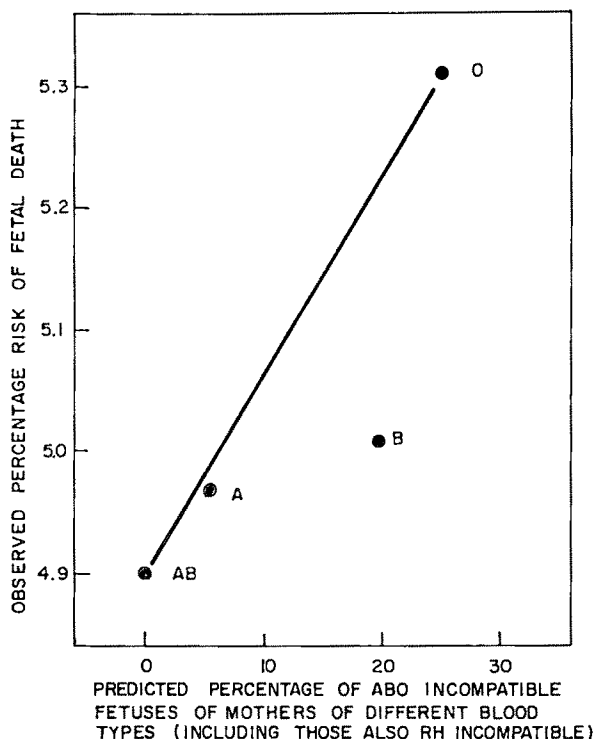


FIG. 2. Observed fetal death risk and predicted ABO incompatibility frequency for mothers of different ABO blood types, excluding cases of combined ABO and Rh incompatibility.

amounting to less than one-third that found in comparisons involving the ABO locus, and is statistically insignificant despite the large numbers of observations. The net importance of major allele differences at the Rh locus would thus seem to have been overestimated in comparison with that of major ABO differences.

INTERACTIONS OF ABO AND RH ALLELES

A belief that the capacity of a mother to eliminate ABO incompatible fetal red cells from her blood stream may serve to protect her against fetal deaths due to simultaneous Rh incompatibility is borne out by detailed comparisons of risks to mothers having different ABO and Rh blood type combinations (table 3). Fetal deaths are most frequent for AB Rh-negative mothers, who may be regarded as most unprotected against Rh sensitization, and least frequent to AB Rh-positive mothers whose offspring are unlikely to be incompatible with respect to either blood group system. The effect is large and statistically significant. Other ABO-Rh maternal constitutions fall in intermediate positions on the scale, although not necessarily in these positions which might be predicted for them.

When mothers below age 25 are excluded, such differences in risks become more striking (table 4) as would be expected since the opportunity for Rh sensitization increases with the number of pregnancies. The AB mothers, when

also in possession of an Rh-positive allele, are exposed to the lowest risk of any of the blood group combinations. Where they lack the Rh-positive allele, however, they become the most vulnerable of all. The difference is large, equivalent to a 66 per cent increase, and is statistically highly significant.

The singular vulnerability of AB Rh-negative mothers is observed consistently at ages from 25 years upward, but is not detected in younger mothers as might be expected where prior sensitization must be involved (table 5). A gradual decline in relative risk with increasing age of mother above age 29 might perhaps be attributed to failure of sensitized mothers to continue having children in the latter part of their reproductive period, a possibility which will be considered again later in another connection.

For mothers of A, B, or O blood type, presence or absence of the Rh-positive gene makes much less difference, amounting to about one-seventh to one-twelfth the effect observed for AB mothers. The degree of protection afforded to Rh-negative mothers by absence of A or absence of B from their own genotypes is

TABLE 3. RISK OF FETAL DEATH BY COMBINED ABO AND Rh TYPE OF MOTHER

Blood Type of Mother	Fetal Deaths	Live Births (10%)	% Fetal Deaths	Relative Incidence	χ^2
AB Rh-neg	199	320	6.22		
O Rh-pos	11,282	21,189	5.32		
B Rh-neg	613	1,152	5.32		
O Rh-neg	2,172	4,152	5.22		
A Rh-neg	1,610	3,162	5.09		
B Rh-pos	3,001	6,064	4.95		
A Rh-pos	7,523	15,232	4.94		
AB Rh-pos	860	1,829	4.70		
<i>Comparisons</i>					
AB, Rh-neg/Rh-pos			6.22/4.70 =	1.32	7.8
B, Rh-neg/Rh-pos			5.32/4.95 =	1.08	1.9
A, Rh-neg/Rh-pos			5.09/4.94 =	1.03	.8
O, Rh-neg/Rh-pos			5.22/5.32 =	.98	.5

TABLE 4. RISKS OF FETAL DEATH TO OLDER MOTHERS OF DIFFERENT BLOOD TYPES

Blood Type of Mother	Fetal Deaths	Live Births (10%)	% Fetal Deaths	Relative Frequency	χ^2
<i>Mothers age 25 and over</i>					
AB Rh-neg	164	200	8.20	1.66	18.9
AB Rh-pos	618	1,247	4.95		
B Rh-neg	459	756	6.07	1.09	1.7
B Rh-pos	2,152	3,860	5.58		
O Rh-neg	1,621	2,647	6.37	1.06	2.8
O Rh-pos	8,211	13,615	6.03		
A Rh-neg	1,174	2,020	5.83	1.05	1.5
A Rh-pos	5,512	9,969	5.54		

thus not so very different from that enjoyed when both are absent simultaneously. This might seem, superficially, to suggest a capacity on the part of type A mothers to get rid of fetal red cells of type A that have entered the maternal blood stream (as well as to get rid of those of type B) and a corresponding capacity of type B mothers to get rid of fetal cells of type B, and for each to do so with almost as much dispatch as would a mother of blood group O. Such responses are inherently unlikely, however, and an alternative explanation must be sought.

The same sort of comparison may be made the other way round. Among Rh-positive mothers the risk of fetal death is lowest when A and B are both present in the maternal genotype. An increase of 12 to 13 per cent is observed when either A or B is absent, and of 22 per cent when both are absent simultaneously (table 6). The increases in risk for the two types of absence are thus almost strictly additive. The same conclusion cannot be drawn, however, for Rh-negative mothers. Among these, the simultaneous absence of the A and the B

TABLE 5. EFFECT OF MATERNAL AGE ON RELATIVE FREQUENCY OF FETAL DEATHS TO AB Rh-NEGATIVE AND AB Rh-POSITIVE MOTHERS

Age Group of Mother	AB Rh-negative Mothers		AB Rh-positive Mothers		Relative Frequency	χ^2
	Fetal Deaths	Live Births (10%)	Fetal Deaths	Live Births (10%)		
0-19	8	20	39	106	1.09	
20-24	27	100	203	476	.63	
25-29	65	98	227	637	1.86	
30-34	54	70	206	432	1.62	
35-39	34	27	138	149	1.36	
40-49	11	5	47	27	1.24	
Weighted mean (all ages)					1.34	8.0
Weighted mean (ages 25 and up)					1.65	17.9

TABLE 6. EFFECT OF ABSENCE OF ABO BLOOD FACTORS IN Rh-NEGATIVE AND Rh-POSITIVE MOTHERS AGE 25 AND OVER ON THE RISK OF FETAL DEATH

ABO Factor Missing from Mother	Risk of Fetal Death (per 100 live births)	Difference as Compared with "Neither Missing"	% Change in Risk
<i>Rh-negative mothers</i>			
neither missing	8.20	—	—
A missing	6.07	-2.13	26 % decrease
B missing	5.82	-2.38	30 % decrease
both missing	6.37	-1.83	22 % decrease
<i>Rh-positive mothers</i>			
neither missing	4.96	—	—
A missing	5.58	+0.62	13 % increase
B missing	5.54	+0.58	12 % increase
both missing	6.03	+1.07	22 % increase

allele has, if anything, less effect (in this case observed as a reduction in risk) than absence of either allele alone.

The source of the anomaly in both kinds of comparison may be identified as associated almost wholly with the AB Rh-negative maternal phenotype. When all other maternal phenotypes are arrayed in order of risk of fetal death, this risk is found to rise smoothly with the number of antigenically active alleles that are unrepresented in the maternal genotype (Fig. 3) although there is no apparent theoretical reason why this should be so. Only the AB Rh-negative blood type fails to fit neatly into the scheme so as to appear as a special case.

The known interactions between the ABO and Rh systems might, at first sight, seem adequate to account for the relationships apparent in Fig. 3. AB Rh-negative mothers would be most strongly prone to fetal loss from Rh incompatibility, where fathers are unselected for ABO blood type, owing to the absence of possible protection from ABO incompatibilities. AB Rh-positive mothers would not be susceptible to loss from either ABO or Rh incompatibilities. It is difficult, however, to account for the more striking features of the observed relationship.

The expected percentages of fetuses rendered incompatible by the A, B, and Rh-positive genes, and combinations thereof, may be calculated separately for each maternal phenotype from the known gene frequencies (table 7). Such

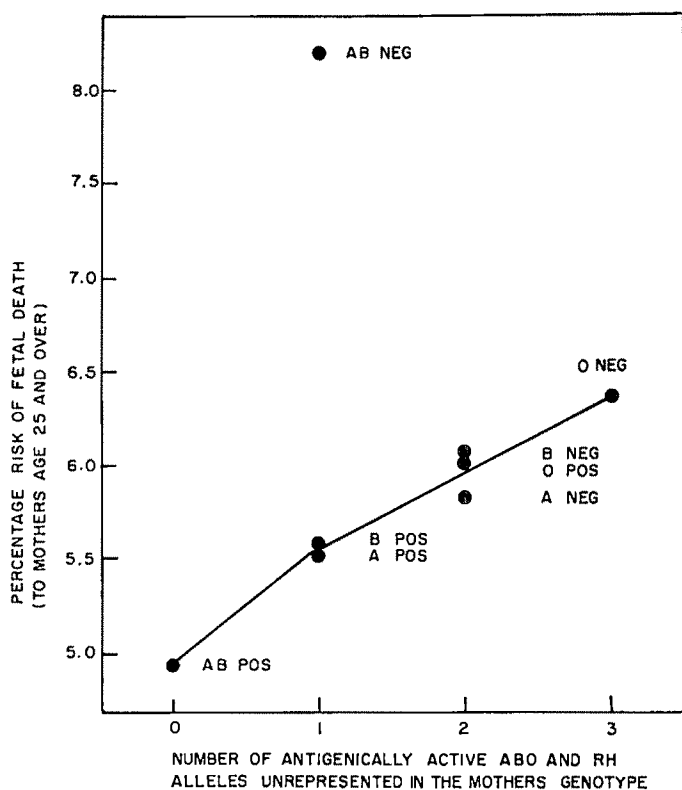


FIG. 3. Effect of absences of antigenically active alleles.

calculations show that for no one sort of incompatibility, and for no simple combination of incompatibilities and protective effects, would one expect either (a) the observed close correlation of risk of fetal loss with number of antigenically active alleles unrepresented in the mother, or (b) the very large difference in risk to AB Rh-negative mothers as compared with other high risk maternal phenotypes.

The proportions of fetuses at risk from either kind of single incompatibility (ABO or Rh) may be plotted in a similar manner, excluding those at risk from double incompatibilities (ABO and Rh) as not being especially vulnerable (Fig. 4). The relative risk to mothers of the various blood types that would be expected on these assumptions are seen to differ widely from the observed risks shown in Fig. 3, in the respects noted above. This is true whether the predictions are based on gene frequencies for whites or for negroes. No set of assumptions concerning the nature of the possible locus and allele interactions has been found that provides anything approaching quantitative agreement between the observed and expected relative risks to fetuses of mothers of the different blood groups.

Differences in allele frequencies associated with various social and racial groups within the population having widely different fetal death rates and opportunities for ascertainment of such deaths might, of course, modify predictions made on simple theory. However, the essential similarity between predictions based on allele frequencies for whites and for Negroes argues against an interpretation in these terms. Added to this, the precision with which observed risk of fetal death is correlated with the number of antigenically active ABO and Rh alleles unrepresented in the mother's genotype (when the AB Rh-negative mothers are excluded), where no such precision is predicted on present theory, would seem hardly to be possible if it were merely fortuitous and secondary to population inhomogeneities of a social or racial kind.

Perhaps the number of antigenically active ABO and Rh alleles unrepresented in the mother's genotype is, in fact, the important underlying variable for all

TABLE 7. EXPECTED PROPORTIONS OF INCOMPATIBLE FETUSES TO WHITE AND NEGRO MOTHERS OF DIFFERENT PHENOTYPES

Race, and Kind of Incompatibility	% of Fetuses Incompatible, by Maternal ABO and Rh Phenotype							
	O Rh- neg.	O Rh- pos.	A Rh- neg.	A Rh- pos.	B Rh- neg.	B Rh- pos.	AB Rh- neg.	AB Rh- pos.
<i>White Mothers¹</i>								
ABO only (single)	12.5	32.6	3.0	7.8	9.5	24.8	0.0	0.0
Rh only (single)	41.6	0.0	56.9	0.0	46.4	0.0	61.7	0.0
ABO and Rh (double)	20.1	0.0	4.8	0.0	15.3	0.0	0.0	0.0
<i>Negro Mothers²</i>								
ABO only (single)	8.5	31.4	3.6	13.3	4.9	28.1	0.0	0.0
Rh only (single)	49.9	0.0	63.1	0.0	59.6	0.0	72.8	0.0
ABO and Rh (double)	22.9	0.0	9.7	0.0	13.2	0.0	0.0	0.0

¹Based on allele frequencies: O = .674, A = .248, B = .078 (Glass and Li, 1953); Rh-negative = .383, Rh-positive = .617 (Sinnott, Dunn and Dobzhansky, 1958).

²Based on allele frequencies: O = .685, A = .181, B = .134 (Glass and Li, 1953); Rh-negative = .272, Rh-positive = .728 (Sinnott, Dunn and Dobzhansky, 1958).

except the high-risk AB Rh-negative mothers. As applied to the two extreme opposite classes of mothers, AB Rh-positive and AB Rh-negative, predictions based on the above view do not necessarily differ from those which would be inferred from a protective effect of ABO incompatibility against Rh sensitization of the mother. Only for the medium-risk mothers of the remaining blood types would predictions based on the two alternative views differ. And, for these mothers, the observed risks of fetal deaths are precisely predictable only on the former view.

The present data do not necessarily conflict with previous findings although they might perhaps appear to do so. For example, Cohen (1960) has shown, by means of an exceedingly thorough statistical analysis, that the frequencies of the different ABO phenotypes deviate significantly from random expectation among sensitized Rh-negative mothers married to Rh-positive husbands, among the husbands of the sensitized women, and among living offspring. In each case, the deviation is in the direction that would be expected if ABO incompatibility of the fetus tends to protect against Rh-sensitization of the mother. There are at least two possible kinds of effect, however, that cannot be excluded by this prior work.

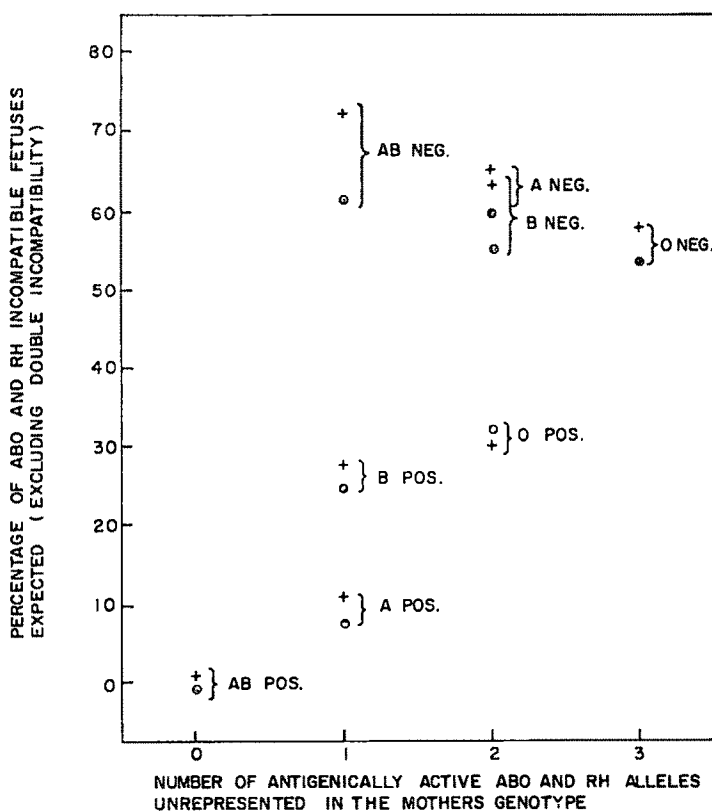


FIG. 4. Frequency of incompatible fetuses expected of white and Negro mothers. Circle = white; Cross = Negro.

First, the analysis carried out by Cohen was not designed to account quantitatively for the relative magnitudes of the various deviations from random expectation, in terms of partial or complete protection of this sort alone, and thus does not wholly exclude a possible disproportionate risk of sensitization among mothers of certain ABO constitutions as compared with others, even where fetal incompatibilities, if any, are of similar kinds.

Second, it is at least a formal possibility that the various ABO and Rh maternal phenotypes might be associated with differences in risk of fetal loss other than those arising out of the known antigenic responses, and perhaps uncorrelated with fetal ABO or Rh constitution. If so, these additional risks would remain undetected with the kinds of information analyzed by Cohen, and could only be satisfactorily studied using ascertainment of actual fetal deaths.

Further discussion of interpretations would not be profitable at this point, especially as the primary purpose of the paper is to emphasize the extent of the present failure to integrate, and to make systematic use of, the large quantities of information on blood groupings, family reproductive events, and social characteristics that are already being recorded routinely.

AGE ANALYSIS — RATIOS OF BLOOD TYPES AMONG
MOTHERS OF LIVEBORN INFANTS AND OF DEAD FETUSES,
FOR DIFFERENT AGE GROUPS OF MOTHER

The special predisposition of AB Rh-negative mothers to lose their fetuses should operate to increase the proportion of Rh-positive blood types among AB mothers who are successful in producing live offspring. This effect is observed and, as would be expected, is all the more striking when mothers below age 25 are excluded (table 8).

Substantially lower proportions of Rh-positives are found among types A, B, and O mothers of liveborn offspring, and the proportion is lowest for type A

TABLE 8. PROPORTIONS OF Rh BLOOD TYPES AMONG MOTHERS OF
DIFFERENT ABO CONSTITUTIONS AND OF DIFFERENT AGE GROUPS

ABO Blood Type of Mother	Rh- Positive Mothers of Liveborn	Rh- Negative Mothers of Liveborn	Ratio pos./neg. (for mothers of liveborn)	Ratio pos./neg. (for mothers of dead fetuses)
<i>All ages of mother¹</i>				
AB	1,829	320	5.71	
O	21,189	4,152	5.27	
B	6,064	1,152	5.26	
A	15,232	3,162	4.82	
<i>Mothers 25 and over²</i>				
AB	1,247	200	6.23	3.77
O	13,615	2,647	5.15	4.70
B	3,860	756	5.10	5.07
A	9,969	2,020	4.94	4.69

¹Data from table 3.

²Data from table 4. The over all ratio of positive/negative is 5.05 as based on combined data for all mothers of liveborn infants and dead fetuses, and allowing for the fact that the data for livebirths represent only a 10 per cent sample.

mothers in whom the risk of fetal death is known to be least affected by presence or absence of the Rh-positive allele (see table 4). This large difference between AB and A mothers is statistically significant and is consistent for all age groups from 25 years up (table 9). The relationships are approximately of the kinds predictable from known ABO-Rh interactions, except that type O mothers would be expected to be more extreme in relation to types A and B.

In view of such observed deficiencies of the Rh-negative allele among mothers of liveborn infants we might expect to find increased numbers among mothers of dead fetuses as compared with the overall ratio. This, however, is only partially true and there is an apparent failure of the two groups of mothers to complement one another. At least three interpretations are possible: (a) Fetal deaths to Rh-negative mothers may tend to be selectively lost to the present study through some substantial fraction of them occurring prior to the usual time at which a mother is blood typed, thus leading to a higher-than-expected ratio of positive/negative mothers of dead fetuses. (b) A substantial under-reporting of all kinds of fetal deaths, irrespective of cause or maternal blood group, might prevent some part of the deficiency of Rh-negative phenotypes which are deficient among mothers of liveborn infants from being represented as an excess among mothers of dead fetuses. (c) Mothers of Rh-negative constitution might tend, where they have experienced prior difficulties from Rh incompatibility, to refrain from further reproduction. The present data provide no rigorous discrimination between these alternatives, although at first sight the second and third will seem more in keeping with previous findings.

This line of reasoning may be pursued quantitatively in the case of AB mothers and some consequences of the various interpretations may be examined. Among mothers of age 25 and over who have had dead fetuses the ratio of Rh-positive/Rh-negative is only 3.77 (*i.e.*, 618/164; see tables 4 and 8) as compared with 6.23 for those who have had live births. The directions of the two deviations from an over all ratio of approximately 5.0 for all mothers irrespective of ABO blood type are as expected, but their magnitudes are not. The shift to a ratio of 6.2 for AB mothers of liveborn infants implies a deficiency of about 33 Rh-negative mothers per thousand among those of AB blood type who have

TABLE 9. PROPORTION OF RH-POSITIVE MOTHERS OF LIVEBORN INFANTS, AMONG THOSE WHO ARE AB AS COMPARED WITH A, BY AGE GROUP OF MOTHER

Age Group of Mother	AB Mothers of Liveborn		A Mothers of Liveborn		Relative Frequency	χ^2
	Rh-pos	Rh-neg	Rh-pos	Rh-neg		
0-19	106	20	839	208	1.31	
20-24	476	100	4,424	934	1.01	
25-29	637	98	5,042	1,019	1.34	
30-34	432	70	3,216	624	1.20	
35-39	149	27	1,391	309	1.23	
40-49	29	5	320	68	1.23	
Weighted mean (all ages)					1.19	7.2
Weighted mean (ages 25 and up)					1.27	8.9

succeeded in having live offspring. Only a small fraction of this deficiency is accounted for by the excess of Rh-negatives found among AB mothers of dead fetuses which, when calculated, is found to be only about 3 per 1,000 live births to AB mothers. Thus, for each thousand live births to AB mothers there is an apparent deficiency of about 33 AB Rh-negative mothers of which only about 3 appear as an excess of Rh-negative constitution among mothers of dead fetuses. If only one of the suggested interpretations were correct we would have to suppose that the thirty who are not accounted for, either (a) lost their fetuses before being blood-typed, (b) represented a failure to report as much as 90 per cent of all fetal deaths, or (c) refrained from reproducing because of previous Rh difficulties. In view of the magnitude of the effect it is altogether possible that more than one of these causes may be operating.

A similar line of argument might be followed using data from other ABO blood types of mothers, but would be less profitable because the differences are small and of limited statistical significance. That A, B and O mothers differ so little from one another, and so much from AB mothers, has been noted earlier in another connection.

Such deficiencies of Rh-negative individuals among mothers of liveborn infants are particularly noticeable in the middle reproductive years (table 10) as is strikingly shown for AB mothers (Fig. 5). Presumably, younger Rh-negative mothers tend not to have been sensitized and so are better represented in the early part of the reproductive period than in the middle. Just why they should again become well represented towards the end of the period is not immediately apparent, although the trend occurs consistently within all ABO blood types. Conceivably, sensitized women may choose to continue reproducing longer in order to achieve desired family sizes, or perhaps there are biological reasons that are still unknown.

Blood types of mothers of dead fetuses do seem to provide a clue (Fig. 6). For those of type AB, in whom such effects are most easily studied, there is a complementary rise in the proportion of Rh-positives in the latter part of the reproductive period. This would argue that the additional Rh-negative mothers who attempt to reproduce at this time in their lives are successful at it and are

TABLE 10. EFFECT OF MATERNAL AGE ON THE PROPORTIONS OF Rh BLOOD TYPES AMONG ALL MOTHERS OF LIVEBORN INFANTS

Age Group of Mother	Rh-pos	All Mothers of Liveborn Rh-neg	Ratio pos/neg	Relative Incidence	χ^2
0-19	2,932	587	5.00		
20-24	12,691	2,556	4.94		
25-29	14,541	2,716	5.36		
30-34	9,009	1,736	5.24		
35-39	4,158	880	4.73		
40-49	893	191	4.67		
<i>Comparison</i>					
25-34	23,640	4,452	5.31		
not in this range	20,674	4,224	4.88	1.09	13.4

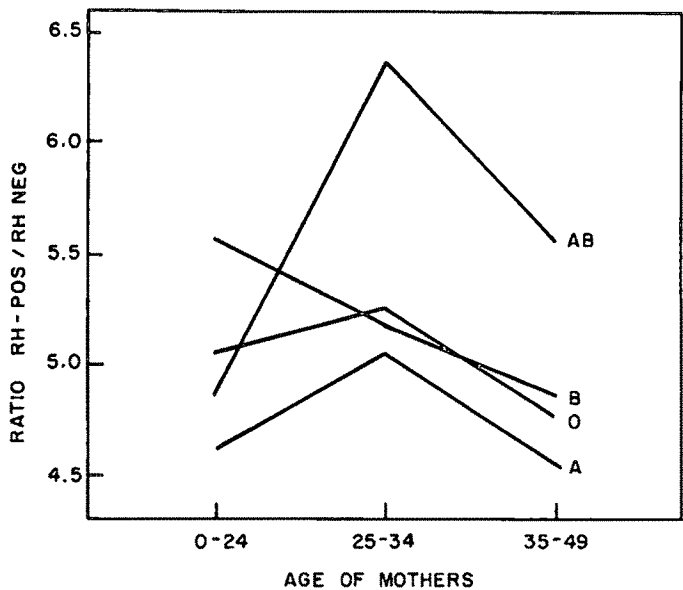


FIG. 5. Age analysis, mothers of liveborn infants.

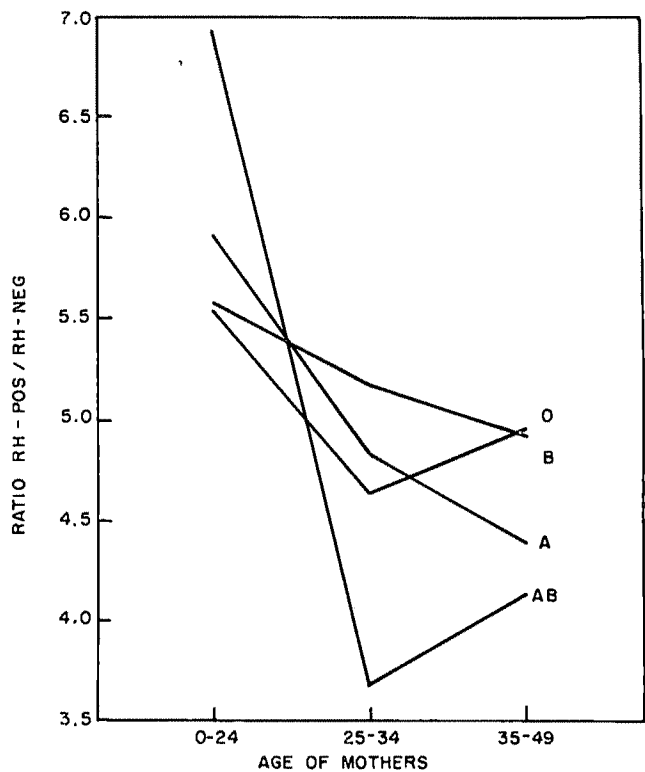


FIG. 6. Age analysis, mothers of dead fetuses.

A Familial Chromosome Variant in a Subject with Anomalous Sex Differentiation

HERBERT L. COOPER AND RAIMOND HERNITS

*Human Genetics Branch, National Institute of Dental Research,
National Institutes of Health, Public Health Service,
U. S. Department of Health, Education, and Welfare, Bethesda, Maryland*

THE SIMPLIFICATION OF TECHNIQUES for the examination of human chromosomes has permitted their application to a wide variety of pathological conditions. In addition to the now well known autosomal trisomic syndromes and the large number of sex chromosome anomalies, there have been reported many isolated cases of numerical or morphological alterations of the karyotype. There is, however, a dearth of information on possible chromosomal variation among the clinically normal population. Since most subjects on whom chromosomal studies are performed are pre-selected from a clinically abnormal population, caution must be exercised in assigning a causal role to any chromosome anomalies found in an isolated case. The subject presented in this report is an example.

CASE MATERIAL

The pedigree of the family to be discussed is shown in Fig. 1. The proband was 20 years old and had been raised as a female. She was seen medically because of primary amenorrhea and failure of secondary sexual development. She was tall, hirsute, muscular, and had no breast development. Axillary and pubic hair were present and masculine in distribution. External genitalia were female in appearance. An enlarged clitoris was present with a urethral meatus at its base, but no vaginal orifice was found. Buccal mucosal smears were sex-chromatin negative.

Bilateral inguinal masses were palpable which on biopsy proved to be undescended testes. No spermatogenesis was seen microscopically in otherwise normal seminiferous tubules. The clinical diagnosis was male pseudohermaphroditism with severe hypospadias.

The proband had a twin sister who was sexually normal. She suffered from epileptiform seizures and was controlled with Dilantin, from which she had developed gingival hyperplasia as a side effect. She was otherwise healthy.

A younger sister, 18 years old, and the mother were both healthy and normally developed females. The father and two older sibs could not be located up to the time of the present report, but were said to be in good health. Both of the older sibs were married and fertile.

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CHROMOSOME STUDIES

Chromosome examination was performed on peripheral blood from the proband, the twin sister, the mother and the younger sister. The method employed was similar to that described by Moorhead *et al.* (1960).

The chromosome number of the proband was uniformly 46 in 60 examined microscopically. The karyotype was the same in all of 1 examined photographically, one of which is shown in Fig. 2. The sex complement was consistent with a normal XY constitution, thus supporting the clinical diagnosis. The autosomes were unremarkable except for pair #1. In all clear metaphases examined, the #1 pair was asymmetrical. One member of the pair had the median centromere placement typical of chromosome #1 (Human Chromosome Study Group, 1960). The other member of the pair was always longer and had a submedian centromere, giving it a superficial resemblance to a #2 chromosome.

In Fig. 3 the #1 and #2 chromosome pairs from 5 cells are shown. The short arms of the atypical #1 are similar to the corresponding arms of the normal homologue, and longer than the short arms of #2. The long arms, however, are increased in length. In many cells the portion of the long arms just distal to the submedian centromere has a characteristic appearance. There is often a lateral separation of the chromatids in this segment, and the arms may curve in that region. The unusual segment often ends in a secondary constriction about one-fourth to one-fifth the way along the long arm. In Fig. 4, the #1 and #2 pairs are represented, with arm ratios plotted against centromere length (expressed as per cent of the total length of the four chromosomes).

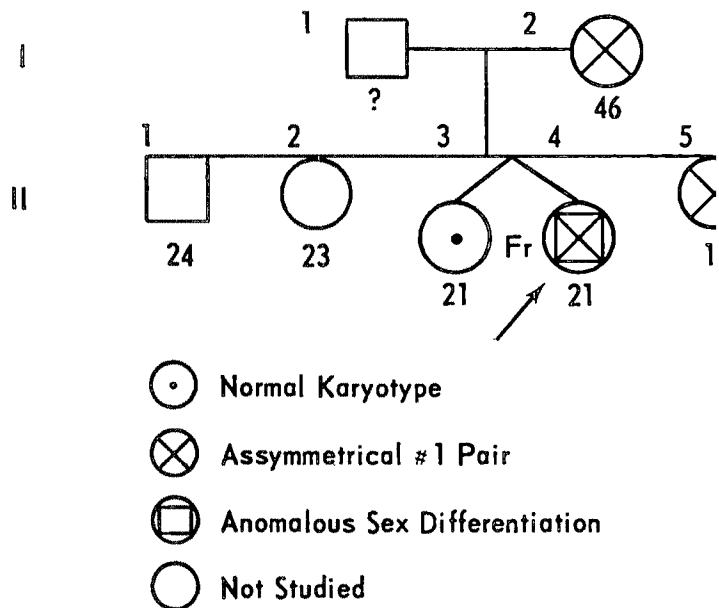


FIG. 1. Pedigree of family with asymmetrical #1 chromosome pair in two generations.

pairs 1 and 2 for each cell). The distribution of points for a normal chromosome pair, #2, may be compared with that of pair #1. The figure shows two clusters for the #1 chromosome and a single one for the #2. There is little overlap among the clusters and it is clear that a group of #1 chromosomes is present which has a length and arm-ratio relationship which are distinct from either the normal #1 or the #2. On the basis of these findings we have concluded that the atypical #1 chromosome has an abnormal segment adjoining the centromere, accounting for its increased length.

It will be observed from Fig. 2, 3 and 4 that chromosome pair #2 shows some tendency toward asymmetry, both in total length and arm ratio. However, this was quite variable and not evident in all cells. The degree of variability of this finding, compared with the quite constant asymmetry of the #1 pair suggests that the former is probably the result of technical variation rather than of true homolog asymmetry. The long arm of chromosome #2 is the longest uninterrupted segment of chromosomal material in the human chromosome set. If it is affected by the same per cent variation in length as are the other chromosome pairs, (due to intrinsic coiling differences and extrinsic technical factors) the asymmetry thus produced may be more apparent to the eye than in other chromosome pairs, and its absolute amount more readily detected with our rather crude techniques of chromosome length measurements.

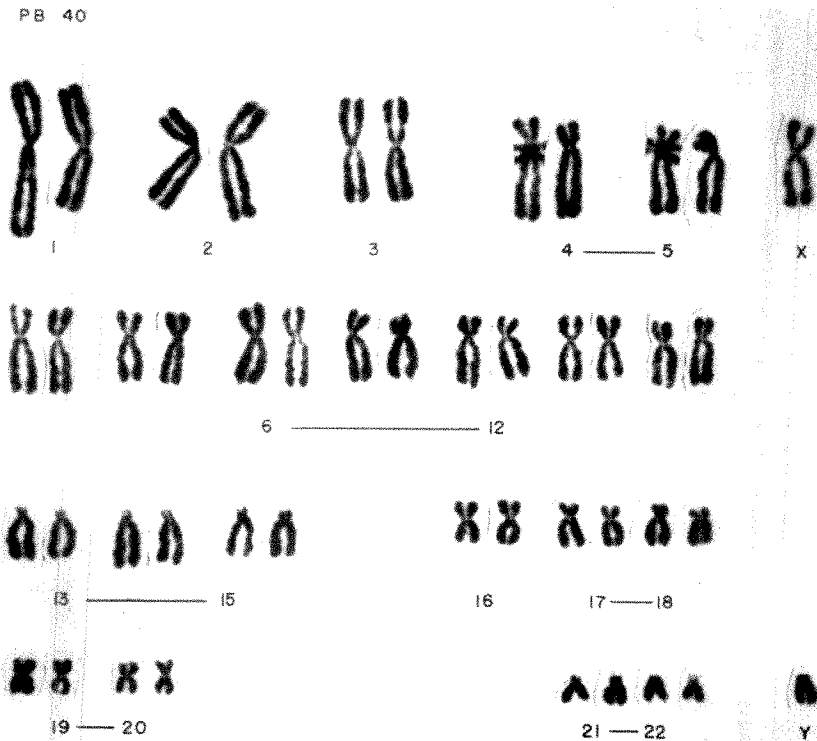


FIG. 2. Karyotype of proband, showing XY sex chromosome constitution and asymmetrical #1 chromosome pair.

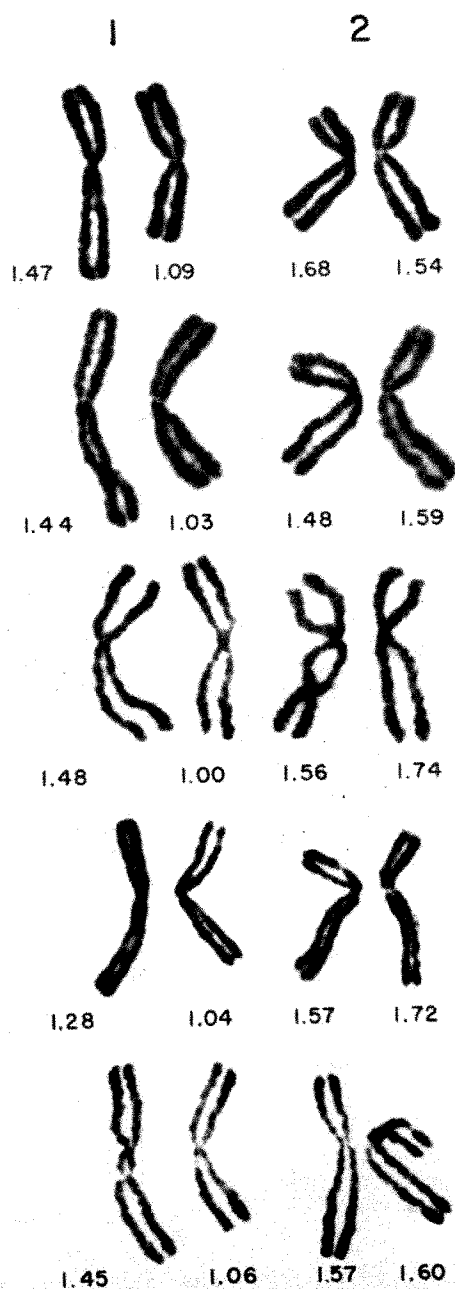


FIG. 3. Chromosome pairs 1 and 2 from 5 cells of the proband, showing asymmetry of the #1 pair. Arm ratios (long/short) are shown below each chromosome. The variant chromosome is mounted as the left-hand member of the #1 pair. Note the region just below the centromere of the variant. It frequently shows diminished separation, thinning, and a prominent secondary constriction.

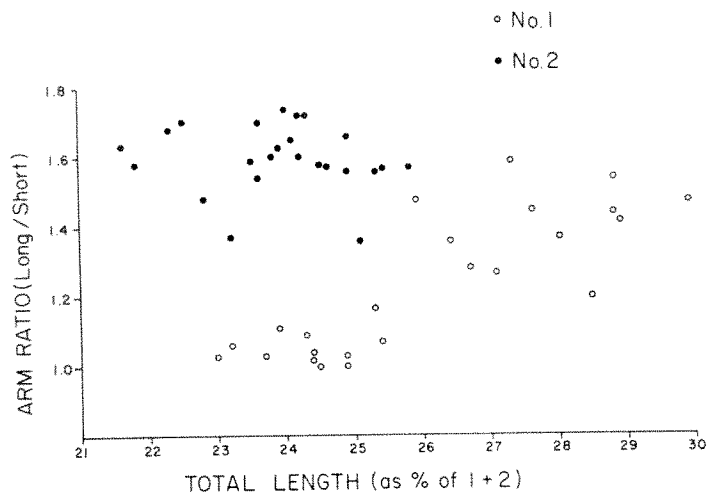


FIG. 4. Plot of measurement data from chromosome pairs 1 and 2 of 12 of the proband's cells. Arm ratios (long/short) are plotted against total length (expressed for each chromosome as per cent. of total length of the #1 and #2 pair for that cell). Note the presence of two separate clusters for the #1 pair, indicating the presence of a group of 1 chromosomes with abnormal arm ratio and increased total length.

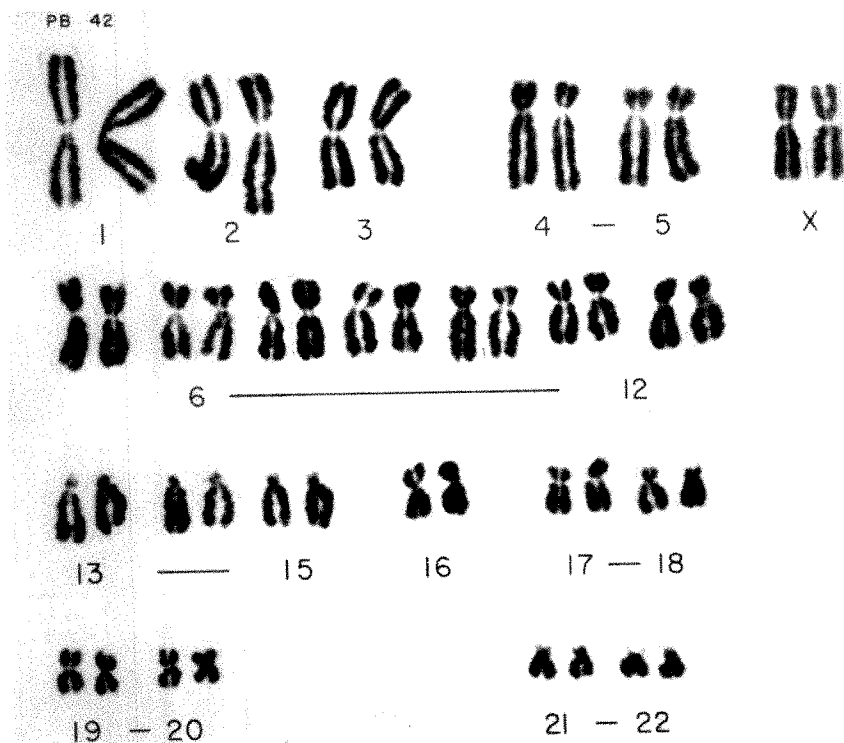


FIG. 5. Karyotype of twin sister of the proband (II-3). Normal female karyotype, normal #1 pair.

The proband's sister had a normal female karyotype (Fig. 5) and did not have any asymmetry of chromosome pair #1. The mother also had a female karyotype (Fig. 6), but in addition, the #1 pair was asymmetrical, with characteristic longer submedian member. The other chromosomes all appeared normal, with no evidence of a deletion or translocation.

The normal younger sister had a karyotype identical to the mother's (Fig. 7) showing the asymmetrical #1 pair and no other abnormalities.

DISCUSSION

The microscopic appearance of the variant #1 chromosome found in members of this family strongly suggests that there is an extra segment in long arm and that it lies near, or adjoins, the centromere. Several possible origins can be postulated for this abnormality. It is most probable that there is an absolute increase in chromosomal material, although we cannot exclude alteration in the coiling structure of an otherwise normal chromosome. Possible sources of extra chromosomal material are reduplication (possibly through unequal crossing-over at meiosis), insertion of a segment of another chromosome during a three-break event, and reciprocal translocation. The last is unlikely in view of the cytological findings, which suggest an atypical segment near centromere.



Fig. 6. Karyotype of proband's mother (I-2). Normal female sex-chromosome constitution. Asymmetrical #1 pair.

It is necessary to decide whether there is sufficient evidence to assert that the proband's anomalous sex development was directly related to the chromosomal abnormality. If unequal crossing over were the source of the extra segment, it would imply that three members of this family each have a #1 chromosome pair containing one normal member and one member with a duplication of the genetic material normally carried on a certain segment of that chromosome. Of three individuals ostensibly carrying the same triple dose of genetic material, only one was clinically abnormal. The reduplication hypothesis, then, appears to be inconsistent with a pathogenic role for the atypical segment. No conclusion can be reached, however, since the proband had an XY sex chromosome constitution and the mother and sister were XX. A sex limitation factor may therefore be involved.

On the hypothesis of an insertion of material from a member of another chromosome pair, it may be argued that the proband was abnormal because she (or he) carried a triple dose of the material on the inserted segment, while the mother and sister were translocation heterozygotes and therefore genetically nearly normal. However, there was no detectable source of the inserted segment in the karyotypes of the mother and sister, and it is unlikely that a segment of such size could be lost from a chromosome without altering its appearance. The only possible candidates for such undetected loss would be the members of the



FIG. 7. Karyotype of proband's younger sister (II-5). Normal female sex-chromosome constitution. Asymmetrical #1 pair.

X-6-12 group. The deletion of a segment of such size from one of the larger members of this group would put its total length in the range of the smaller members of the group. However, if the deletion were from the short arm, the chromosome would be easily detectable as an abnormal acrocentric. If it were from the long arm, the remaining short arms would probably appear too long for any normal member of the smaller range of this group, although it might be mistaken for a member of the pair we usually place as #11. This pair is one of the shortest of the 6-12 group and has a centromere placement distinctly more median than the other pairs in this size range. The presence of another such chromosome would probably be detected. Were the deletion to occur in one of the smaller members of the 6-12 group, its total length would be reduced out of the range of this group altogether, and it would certainly be detected. Thus, the translocation heterozygote theory does not seem to be tenable as an explanation of the normality of the mother and younger sister, and the situation reduces to the one just considered for reduplication.

One attractive hypothesis is that the extra segment is part of an X chromosome which, in the presence of an XY sex chromosome constitution, might affect sex differentiation. In the presence of an XX sex chromosome constitution, it would produce a partial triplo-X, a condition probably compatible with normal female development. Some minimal negative evidence can be derived from the fact that the XY-partial X condition postulated for the proband would be expected to produce something akin to the Klinefelter syndrome, rather than anomalous development of the external genitalia. Such intersexes, when found to have chromosomal aberrations, are most often cases of mosaicism. Sex chromosome mosaicism cannot be ruled out in the present case, since only peripheral blood has been studied.

It must be concluded that, in the absence of a series of similar cases, no decision is justifiable regarding a causal relationship between the chromosomal and clinical abnormalities seen in the proband. It is possible that this type of unusual #1 chromosome is a variant within the range of normal and is found occasionally in the general population. Its occurrence in the proband may be entirely fortuitous.

Another unresolved problem is the relationship, if any, between the subject's disorder and the fact that she was a member of a dizygous twin pair of unlike sex. This situation suggested the possibility of *in utero* circulatory communication between the twins, with resultant chimerism. However, blood group studies failed to reveal any evidence of chimerism, and no chromosomal mosaicism was found in peripheral blood.

The presence of an easily identifiable chromosome marker in this family suggested the possibility of linkage studies using well defined genetic markers. Extensive blood grouping studies and haptoglobin determinations (table 1) have thus far shown the mating to be uninformative for all markers tested with the exception of the Fy^a (Duffy) blood group. For that blood group the findings are consistent with, but in no sense proof of, presence of the Duffy locus on chromosome #1.

The cytogenetic findings in this family are very similar to those reported by

TABLE 1. BLOOD GROUP AND HAPTOGLOBIN PHENOTYPES OF AVAILABLE FAMILY MEMBERS

Subject	Variant #1	Blood groups										Haptoglobin
		ABO	MNS	P	Rh	K	k	Kp ^a	Kp ^b	Fy ^a	Jk ^b	
I-2	+	A ₁	Ms	+	Rh ₁ Rh ₁	—	+	—	+	+	—	1-1
II-3	—	A ₁	MSs	+	Rh ₁ Rh ₁	—	+	—	+	+	+	2-1
II-4	+	A ₁	MNss	+	Rh ₁ rh	—	+	—	+	—	+	2-1
II-5	+	A ₁	MSs	+	Rh ₁ rh	—	+	—	+	—	+	2-1

Subject designations are those shown in Fig. 1.

Patau *et al.* (1961) in a mother and daughter with the oral-facial-digital syndrome (OFD). In both of their subjects an atypical #1 chromosome was present which appeared to resemble somewhat the one described by the authors. They stated confidently that the unusual segment was an inserted segment of another chromosome and that this other chromosome was one they call a member of the 6 - 12 group having a prominent secondary constriction. The authors described the varying microscopic appearances of the secondary constriction region of C', calling it unique in the human chromosome set. Since the unusual segment of the atypical #1 chromosome had the same microscopic appearance, they concluded that it was, in fact, genetically identical to the region mentioned, having been inserted during a three-break translocation. This identification rested solely on the similarity of microscopic appearance since no evidence of deletion was present in either of the subjects, both of which had two normal C' chromosomes. The authors then asserted that both subjects were therefore, trisomic for the secondary constriction region of C'.

We feel that such cytological observations, in the absence of a demonstration of a deletion in another chromosome, do not justify such a positive identification of the source of the inserted segment. In fact, the authors have not satisfactorily demonstrated that the variant #1 chromosome is due to an insertion at the secondary constriction region of the C' chromosome.

Patau *et al.* further concluded that the postulated partial trisomy of the secondary constriction region of the C' chromosome is the cause of the oral-facial-digital syndrome in the mother and daughter they studied. This contention was based on the following reasoning:

"The OFD syndrome is very rare and so is a recognizable insertion in the present case being apparently the first to be described. Evidently, the co-occurrence of the disease and the insertion in mother and daughter cannot be ascribed to chance."

This reasoning ignores the fact that the index subject of this mother-daughter pair was undoubtedly selected for chromosomal study because of the presence of the OFD syndrome, thus rendering its rarity meaningless. The fact that no additional cases were studied and had no detectable chromosomal abnormality instead of leading the authors to modify their contention, was explained by attributing them to a partial trisomy too small to be detected.

It is our opinion that such conclusions regarding causal relationships cannot be made from the type of evidence presented, as we have tried to show in our own material. Such difficulties arise, in part, because of our lack of information regarding the incidence of the chromosome variant in question among a clinically normal population. In the case of the asymmetrical #1 chromosome pair, a recent report by Yunis and Gorlin (1963) is of interest. They described a family, ascertained through a subject with cysts of the jaw, multiple basal cell carcinomata and bifid ribs. The index subject, as well as several affected members of her family, had a #1 chromosome pair indistinguishable from the asymmetrical pair found in our material. They reported another family in whom the clinical syndrome was present without any chromosomal abnormality. In this case the authors quite properly concluded that the chromosomal abnormality was probably unrelated to the clinical syndrome.

It appears then, that asymmetry of the #1 chromosome pair may

definite place as one of the chromosomal variants to be found occasionally among the clinically normal population. The use of such a uniquely marked human autosome in genetic studies is obvious, and an effort should be made to determine the frequency of its occurrence through the accumulation of a large, reliable series of karyotypes on a representative sample of the normal population.

SUMMARY

Chromosome studies on a male pseudohermaphrodite revealed an XY sex-chromosome constitution and an asymmetry of the #1 chromosome pair. Study of the subject's fraternal twin revealed a normal female karyotype, consistent with her physical appearance. Study of the normal mother and younger sister revealed an XX sex-chromosome constitution and asymmetry of the #1 chromosome pair as in the proband. The asymmetry was due to the apparent presence of a segment of additional chromosomal material adjacent to the centromere of one member of the #1 pair. The other member appeared normal.

The nature of the chromosomal lesion is discussed, and it is concluded that no decision can be made as to any causal relationship between the chromosomal and clinical abnormalities in the proband. In view of this and other reports of a similar nature, it is suggested that asymmetry of the #1 chromosome pair may be a variant which occurs occasionally among the clinically normal population.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Dr. Margery Shaw, University of Michigan Medical School, Department of Human Genetics, for making available to us the facilities of her laboratory while obtaining specimens from members of the family reported here.

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A Method for Calculating the Inbreeding Coefficient

II. Sex-Linked Genes

AKIO KUDÔ AND KOJI SAKAGUCHI

*Mathematical Institute, Faculty of Science,
Kyushu University, Fukuoka, Japan*

A PREVIOUS ARTICLE (Kudô, 1962) presented a new method for calculating the inbreeding coefficient for autosomal genes. It is the purpose of this paper to extend this method to genes borne on the X-chromosomes. The coefficient of inbreeding for genes of the latter variety is defined as the probability that a human female will possess at a given genetic locus on the X-chromosome two genes identical in their origin. The difference in number of X-chromosomes between normal males and normal females makes this notion applicable to females only.

The terms ancestral line, common ancestor, joining and loop were defined in the earlier paper, and are used here in the same manner. Since, normally, sex-linked genes are not transmitted from a father to his son, a loop containing the relation "son-father" does not contribute to the inbreeding coefficient under consideration. Thus, a loop or an ancestral line is said to be pertinent to the inbreeding coefficient for sex-linked genes if it does not contain relations of the type "son-father." The inbreeding coefficient for sex-linked genes can be calculated by the following formula (Haldane and Moshinsky, 1939; Wright, 1951):

$$F' = \sum \frac{1}{2^{m_1+m_2+\delta}} (1 + F_0')$$

where summation extends over all pertinent loops; m_1 and m_2 are the number of relations of the type "child-mother" in the paternal and maternal lines of the loop, respectively; δ is equal to zero or one depending upon whether the common ancestor of the loop is male or female; and F_0' is the inbreeding coefficient of the common ancestor of the loop, where F_0' is taken to be zero when the common ancestor is a male.

PEDIGREE SHEET FOR SEX-LINKED GENES

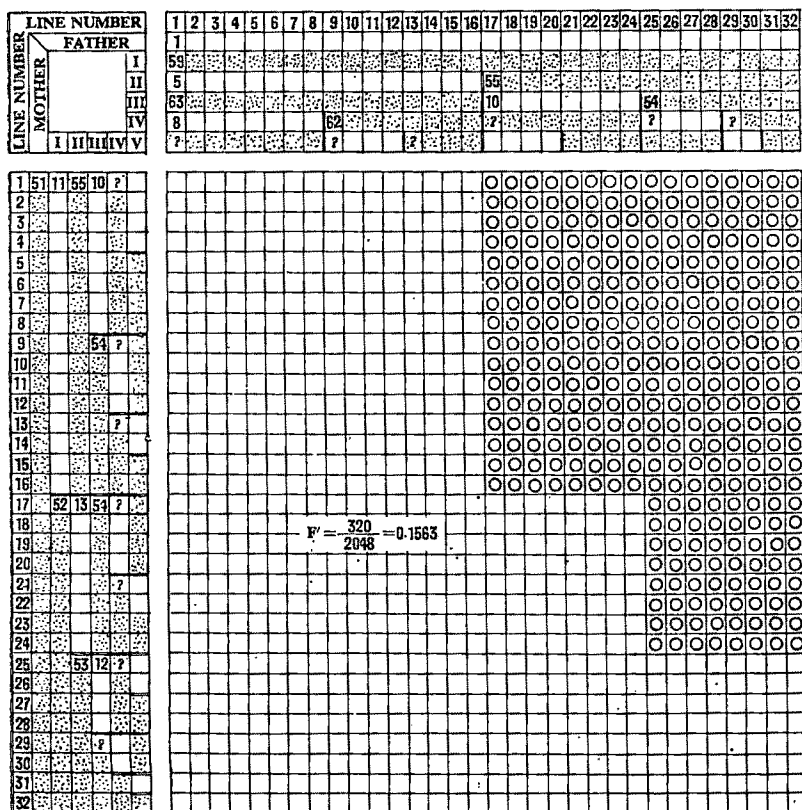
To calculate the inbreeding coefficient for sex-linked genes we suggest a pedigree sheet of the type shown in Fig. 1 and 2. The difference between this pedigree sheet and the one for autosomal genes lies only in shadowing. This modification is sufficient to ensure the exclusion of all ancestral lines not perti-

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nent to the inbreeding coefficient. If a given ancestor is a male, the pertinent ancestral lines passing through him pass, in turn, only through his mother; whereas if the given ancestor is a female, the pertinent ancestral lines can pass either through her father or her mother.

Fig. 1 is a diagrammatic representation of the inbreeding coefficient for sex-linked genes of the person in the pedigree of Fig. 1 of the previous paper (Kudô, 1962) assuming that person to be a female. Ancestors 5 and 55 contribute the same number of paternal lines, 1-16 and 17-32, respectively, indicating that the gene coming through the father, 1, from the grandmother, 59, could have come from 5 or 55 with equal probability. Ancestor 54 contributes one-fourth of the paternal lines, 25-32, corresponding to the fact that the probability the gene was derived from 54 is one-fourth. If we consider, now, the maternal lines, we observe that the probability that the gene coming through the mother, 51, had origin 54 through 55 is one-half, whereas the probability the gene came from 54 but not through 55 is one-fourth. In this case the common ancestors are, as noted in the previous paper, 6, 63, 5, 10, 54, and 55, but only the latter two are pertinent for sex-linked genes. All parental lines linking 6, 63, 5, and 10 to the individual whose coefficient of inbreeding is being determined involve the relation son-father. As in the case of autosomal genes, we inscribe

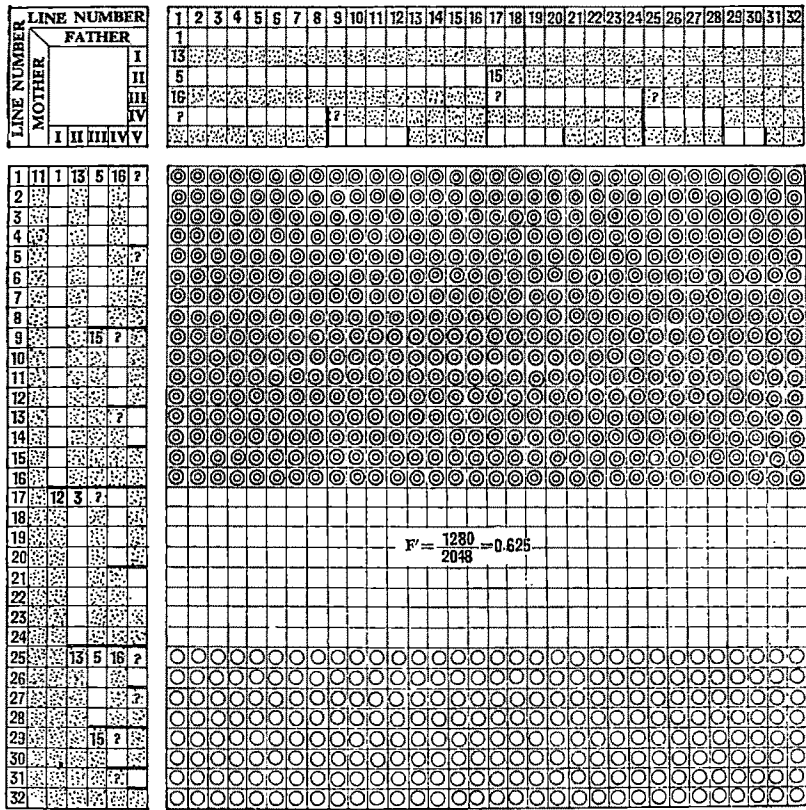


circles in those cells (Fig. 1) where the maternal and paternal line pair contain a common individual. All loops not pertinent to the inbreeding are automatically excluded by the shadowing. The circles occupy 320 of the $32 \times 32 = 1,024$ cells. The ratio $320/1024$ represents the probability that the two genes have the same origin. Since both of the pertinent common ancestors are females, and each has two sex-linked genes, the probability that the two genes of the individual in question are identical is one-half of $320/1024$. Thus, we have the same formula as in the case of autosomal genes

$$F' = \frac{\text{number of circles}}{2(\text{total number of cells})}$$

(A)

In Fig. 1 all of the common ancestors are females, but if one or more of the common ancestors are males, an adjustment in the above formula would be needed, for males have only one X-chromosome. Fig. 2, constructed from the pedigree, appearing as Fig. 3 of the previous paper, serves to illustrate the adjustment. In this case the common ancestors to be taken into account are 1 and 13; the others, 6 and 16, are automatically excluded as the loops associated with them are not pertinent. Now, we observe that the probability of two genes having their origin in individual 1 is one-half, and since 1 is a male this is also



the probability that the genes are identical and derived from 1. With respect to 13, we note that since this individual is a female, the probability that the two genes are identical and derived from 13 is one-half of the probability that they are both derived from 13. This difference in probability can be indicated by inscribing double circles in the cells of male ancestors and counting them as twice the value of single circles. The parents of 1 have common ancestors 6 and 16, but since 1 is a male, 6 and 16 may be disregarded. Thus, we need consider rejoining only in the event the common ancestor is female. Formula A is, thus, still valid, and we have the inbreeding coefficient $1280/2048 = 0.625$.

If rejoining had to be considered, the pertinent loops stemming from the rejoining could be scored, as in the case of autosomal genes, in the lower left portion of the rectangle formed by the loops through which joining occurs. We can inscribe triangles or double triangles inside circles depending upon whether the common ancestor through whom rejoining occurs is male or female.

Exclusion of loops of rejoining through males is the equivalent of the exclusion of rectangles of double circles, and in the case of triple joining, of double triangles. If we count twice the number of double circles, double triangles, and double small circles, we have the formula

$$F' = \frac{\text{circles} + 2 \text{ triangles} + 2^2 \text{ small circles} + \dots}{2(\text{total number of cells})} \quad (\text{B})$$

This expression is identical to the one associated with the inbreeding coefficient for autosomal genes, and can be proved similarly.

The proofs of formulae A and B given here differ from the proofs in the previous paper. The present method is also applicable to the former case and gives, perhaps, a better insight into the probabilistic nature of the coefficient of inbreeding.

SUMMARY OF THE PROCEDURE

1) Enumerate all of the paternal and maternal lines, male unshadowed and females shadowed, as in the case of autosomal genes. It should be noted that fathers of males ancestors are automatically excluded.

2) In every cell corresponding to an ancestral line pair with a common member inscribes a circle if the youngest common member is a female or a double circle if the youngest common member is a male.

3) Rectangles of loops can be identified in the same manner as in the case of autosomal genes, and instances of rejoining can be determined by examining the lower left portion of the rectangles associated with female common ancestors only.

4) Within such rectangles inscribe triangles or double triangles in those cells, if any, where a common ancestor, male or female, occurs.

5) Proceed to inscribing small circles or double small circles if necessary, in the same manner.

6) Count the number of circles, triangles and small circles, and also the double circles, etc. Double each of the latter and add to the former. Apply

formula (A), or, if rejoining or triple joining occur, formula (B) to obtain the coefficient.

A METHOD OF CHECK

In the previous paper we proposed a check to eliminate the possibility that all pertinent loops might not be detected. With a slight modification in the definitions of p and m , the check can also be used in the present case. We define, now.

p = number of known ancestors in the paternal lines
 = 33 minus the number of ? in the paternal lines
 m = number of known ancestors in the maternal lines
 = 53 minus the number of ? in the maternal lines,

and the identity

pm = number of blocks

is still valid.

The constants, 33 and 53, are determined by the size of the pedigree sheets of Fig. 1 and 2, and depend upon the extensiveness of the pedigree. In general, for a pedigree extending through t generations from the parents, the constants are determined by the recurrence relation

$$n(t) = n(t-1) + n(t-2) + k$$

with the initial condition $k = 1$, $n(0) = 1$, $n(1) = 2$ and $k = 2$, $n(0) = 1$, $n(1) = 3$ for p and m , respectively. Some values of these two constants are as follows:

generations	parent	1	2	3	4	5	6
male	1	2	4	7	12	20	33
female	1	3	6	11	19	32	53

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The Linkage Relations of Hemophilia A and Hemophilia B (Christmas Disease) to the Xg Blood Group System

S. H. DAVIES,¹ JUNE GAVIN,² K. L. G. GOLDSMITH,³ J. B. GRAHAM,⁴
JEAN HAMPER,² R. M. HARDISTY,⁵ J. B. HARRIS,⁶ C. A. HOLMAN,⁷
G. I. C. INGRAM,⁸ T. G. JONES,⁹ L. A. McAFEE,¹⁰ V. A. McKUSICK,¹¹
J. R. O'BRIEN,⁶ R. R. RACE,² RUTH SANGER,² AND
PATRICIA TIPPETT²

¹Haematology and Blood Transfusion Units, Royal Infirmary, Edinburgh.

²M. R. C. Blood Group Research Unit, The Lister Institute, London.

³M. R. C. Blood Group Reference Laboratory, London.

⁴Department of Pathology, University of North Carolina, Chapel Hill.

⁵Department of Haematology, The Hospital for Sick Children, Great Ormond Street, London.

⁶Portsmouth and Isle of Wight Area Pathological Service, Portsmouth.

⁷Group Laboratory, Lewisham.

⁸Louis Jenner Laboratory, St. Thomas' Hospital and Medical School, London.

⁹Department of Haematology, The Westminster Hospital, London.

¹⁰The Children's Convalescent Home and School, West Kirby, Cheshire.

¹¹The Moore Clinic, The Johns Hopkins Hospital, Baltimore.

SOON AFTER THE RECOGNITION of the X-linked blood group system called Xg (Mann *et al.*, 1962) some of us reported that in the first seven hemophilia A families to be tested the number of recombinants suggested that the Xg groups were very unlikely to be of use in detecting carriers of the hemophilia A gene (O'Brien *et al.*, 1962). The present paper reports progress in an attempt to establish the recombination fraction between the Xg genes and the genes for hemophilia A (factor VIII deficiency) and for hemophilia B (Christmas disease, factor IX deficiency). The genes for hemophilia A and for hemophilia B occupy separate loci which are not close to each other: the argument for this by Whitaker, Copeland and Graham (1962) depends on the correctness of the present belief that the genes for deutan and for protan color vision are alleles or are fairly closely linked.

METHODS

So many laboratories were involved in the diagnosis of hemophilia A and B that no details of the various methods used can conveniently be given here, but references to local techniques are, when available, made in the legends to the pedigrees. The anti-Xg^a serum used was that of the original donor, Mr. And. of Grand Rapids; it reacts only by the antiglobulin method. Samples of

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blood were taken from families known to have hemophilia A or B and often selected for having as many boys as possible. The samples were sent to London for Xg grouping.

CLASSIFICATION OF SEVERITY OF THE HEMOPHILIA

In case the grades of severity should later be found to reflect heterogeneity for genetic loci, the families have been classified as (1) severe and (2) moderate or mild, according to the following criteria. *Severe*. Spontaneous

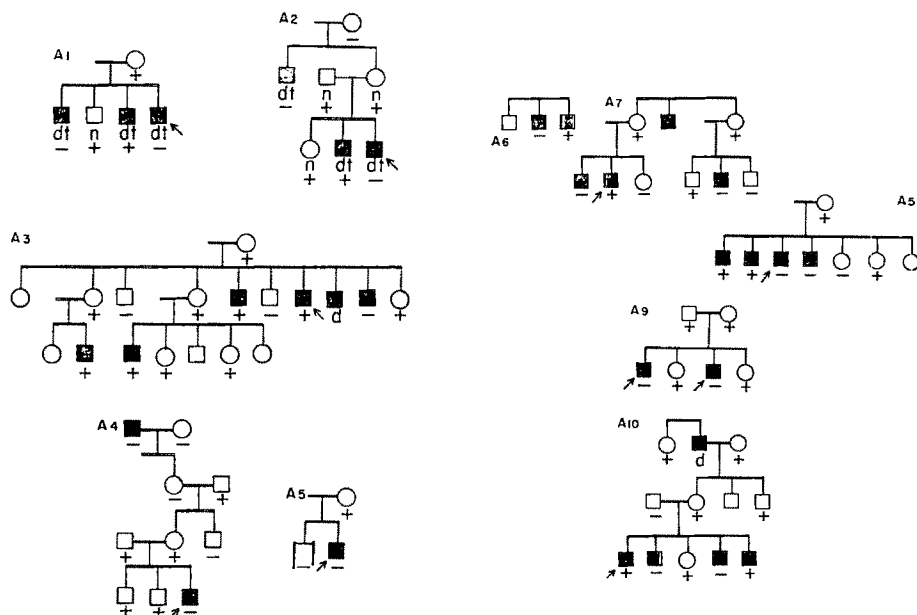


FIG. 1. Hemophilia A and the Xg blood groups: families giving linkage information. Black = hemophilia A, hollow = normal. + = Xg(a+), - = Xg(a-). In families in which color blindness has been detected, dt = deutan, n = normal color vision. Arrow = propositus. d = dead.

Families

- A1 (Hob.) Dr. Graham, U. of N. Carolina. Mild. Part of large Kindred H (Whittaker *et al.*, 1962).
 A2 (Hon.) Dr. Graham, U. of N. Carolina. Mild. Part of large Kindred H (Whittaker *et al.*, 1962). Methods: Whittaker *et al.*, 1962.
 A3 (McN.) Dr. Davies, R. Inf. Edinburgh. Severe. Methods: Biggs *et al.*, 1955.
 A4 (Wes.) Dr. McKusick, Johns Hopkins. Mild. Negro family. Methods: Margolius *et al.*, 1961.
 A5 (Sta.) Dr. Ingram, St. Thomas' Hosp. Med School. Moderate. History of 'bleeders' in mother's family. Methods: Biggs, 1957.
 A6 (Fie.) Dr. McAfee, Children's Conv. Home, W. Kirby. Severe.
 A7 (Law.) Dr. Jones and Dr. Goldsmith, Westminster Hosp. Moderate. Methods: Biggs and Douglas, 1953.
 A8 (May.) Dr. O'Brien, Cent. Lab., Portsmouth. Mild.
 A9 (Mar.) Dr. O'Brien, Cent. Lab., Portsmouth. Moderate. Two propositi.
 A10 (Att.) Dr. O'Brien, Cent. Lab., Portsmouth. Mild. Methods: Biggs and Macfarlane, 1962.

hemorrhages and prolonged post-traumatic hemorrhages. Plasma factor VIII (anti-hemophilic factor, AHF) less than about 5 per cent. Factor IX (plasma thromboplastin component, PTC, Christmas factor) usually under 1 per cent. *Moderate or mild.* Prolonged post-traumatic hemorrhage only. AHF about 5 to 30 per cent. PTC usually above 1 per cent.

THE Xg GROUPS OF HEMOPHILIA A FAMILIES

During the period covered by this report, 35 hemophilia A families, including 245 members, were tested with anti-Xg^a. The segregation of Xg was such that 10 families gave useful information about linkage. For reasons of space only these 10 families are shown in Fig. 1 and only the more informative branches of these families.

Linkage Calculations

The data were analyzed by the application of the lod scores of Morton (1955) as instructed by Maynard-Smith, Penrose and Smith (1961). The lod scores are shown in table 1, and in Fig. 2 are plotted the antilogs of the sums of these scores which represent the relative probability of θ , the recombination fraction. A correction introduced at the foot of table 1 will be explained below.

From the probability curve the estimate of θ is 0.40, but the estimate has

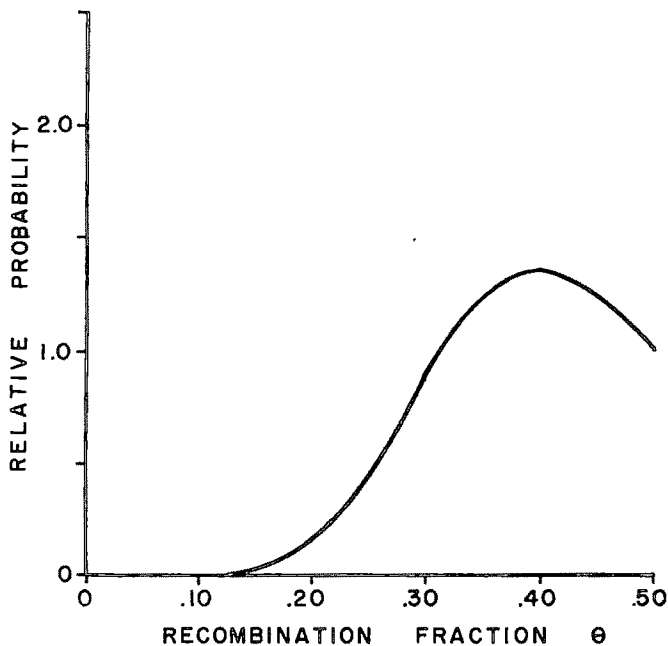


FIG. 2. Xg and hemophilia A: the relative probabilities of various recombination fractions. Horizontal scale = recombination fraction, θ . Vertical scale = antilogs of the observed lod scores, or relative probabilities (table 1). The estimate of θ is, with wide confidence limits, 0.40.

TABLE 1. X_g AND HEMOPHILIA A: THE LOD SCORES OF THE FAMILIES IN FIG. 1

	recombination fraction, θ									
	.05	.1	.15	.2	.25	.3	.35	.4	.45	.499
A1 z_1 3:1, e_1 3:1	-.483	-.241	-.126	-.064	-.030	-.012	-.003	-.001	-.000	0
A2 1 n-rec:1 rec	-.721	-.444	-.293	-.194	-.125	-.076	-.041	-.018	-.005	0
A3 z_1 4:1, e_1 3:2	-.199	.010	.089	.116	.112	.091	.061	.030	.008	0
A4 3 n-rec	.837	.765	.690	.612	.528	.438	.342	.237	.123	0
A5 z_1 1:1, e_1 1:1	-.825	-.528	-.358	-.243	-.160	-.099	-.054	-.024	-.005	0
A6 z_1 1:1, e_1 2:0	-.584	-.340	-.215	-.138	-.087	-.052	-.028	-.012	-.003	0
A7 z_1 1:1	-.721	-.444	-.292	-.194	-.125	-.076	-.041	-.018	-.004	0
z_1 2:1, e_1 2:1	-.769	-.482	-.321	-.216	-.140	-.086	-.047	-.020	-.005	0
A8 z_1 2:2	-.1442	-.887	-.585	-.388	-.250	-.151	-.082	-.035	-.009	0
A9 z_1 2:0, e_1 2:0	.395	.319	.250	.190	.135	.088	.050	.023	.005	0
A10 z_1 2:2, e_1 4:0	-1.323	-.742	-.482	-.316	-.202	-.121	-.066	-.028	-.007	0
total lod score	-5.835	-3.014	-1.643	-.835	-.344	-.056	.091	.134	.098	0
antilog = relative probabilities of θ			.023	.146	.452	.879	1.233	1.361	1.253	1.0
tentative <i>a priori</i> correction	.049	.050	.039	.078	.109	.156	.277	.338	.542	1.219
corrected sum	-5.786	-2.964	-1.584	-.757	-.235	.100	.318	.472	.640	1.219
antilog = relative probabilities of θ (corrected)		.001	.026	.175	.582	1.259	2.080	2.965	4.365	16.560

wide confidence limits. The lower limit of θ at the 1 in 20 level of probability is about 0.24, a figure obtained by the simple method, recommended to us by Dr. C. A. B. Smith, of counting the graph paper squares below the curve and cutting off from the left hand tail one twentieth of their number.

THE Xg GROUPS OF HEMOPHILIA B FAMILIES

In the period covered by this report, 20 families, involving 331 members, were tested with anti-Xg^a and 11 gave information about linkage. The informative branches of the 11 families are shown in Fig. 3.

Two of the families deserve special mention. In family B1 there are 11 recombinants to one non-recombinant; this disproportion is discussed below. In family B5 hemophilia B, protan color vision and Xg are all segregating: the family shows that whatever the alignment of the genes on the mother's two Xs and whatever the order of the genes on the chromosome, at least one of the sons always has to be a double recombinant. This was the first demonstration of double crossing-over in man (Graham *et al.*, 1962).

Linkage Calculations

The lod score are given in table 2 and the antilogs of their sums are plotted in Fig. 4. A correction introduced at the foot of table 2 is explained below. From the probability curve the estimate of θ is 0.5 and the lower limit of θ at the 1 in 20 level of probability is about 0.37.

If the family, B1, with the 11 recombinants to 1 non-recombinant is removed from the calculations, for which the only justification at present is that the segregation in the family is so outrageous, a curve results with a peak corresponding to $\theta = 0.37$ (lower limit at 1 in 20 level = 0.26). This curve, taken from the lod scores in table 2, is included in Fig. 4 (interrupted line) because Dr. J. H. Edwards calculates the probability that the divergence of family B1 from the rest of the families is due to chance to be less than 1 in 600, even after allowing for selection of the most discrepant family. Clearly more hemophilia B families should be grouped.

A PRIORI CORRECTIONS

Dr. J. H. Renwick points out that a correction to allow for the *a priori* probability of linkage between Xg and hemophilia A and between Xg and hemophilia B should be added to the lod scores. The magnitude of this correction depends on the genetical length of the X chromosome and on any assumptions made about interference between cross-over events.

The correction applied to the total lod scores in tables 1 and 2 is based on a total chromosome length of 1.75 morgans (calculated from the data of Ford and Hamerton, 1956) and on the assumptions that nothing is known about the position on the X chromosome of the loci for Xg, hemophilia A or hemophilia B, and that interference is present. The effect of the correction on the estimates of θ and on the confidence limits is shown in table 3.

On the other hand it is possible that the Xg and deutan color-vision loci are

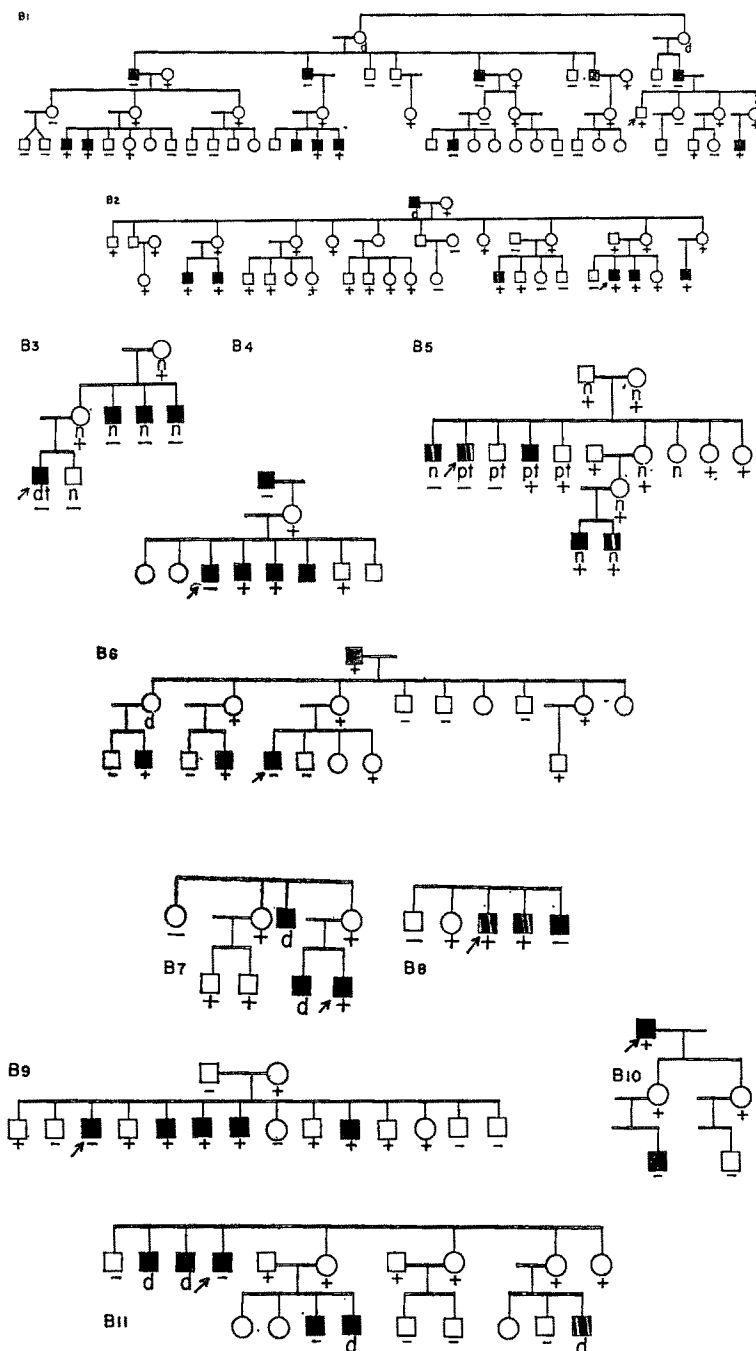


FIG. 3. Hemophilia B and the Xg blood groups: families giving linkage information. Black = hemophilia B, hollow = normal. + = Xg(a+), - = Xg(a-). In families in which color blindness has been detected dt = deutan, pt = protan, n = normal color vision. Arrow = propositus. d. = dead.

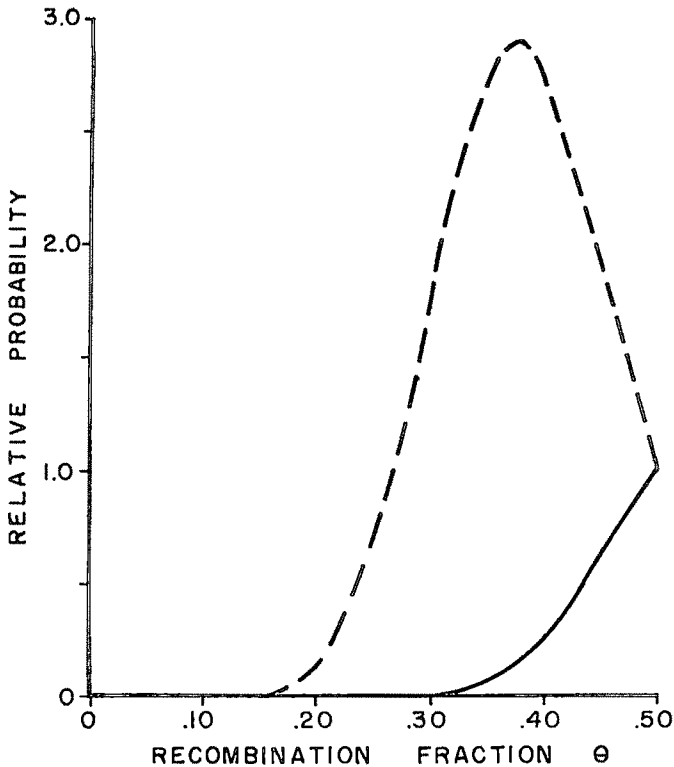


FIG. 4. Xg and hemophilia B: the relative probabilities of various recombination fractions. Horizontal scale = recombination fraction, θ . Vertical scale = antilogs of the lod scores, or relative probabilities (table 2). Continuous curve = antilogs of the observed lod scores, giving an estimate approaching 0.5 for the recombination fraction. Interrupted line = antilogs after removal of family B1 and giving an estimate of 0.37 for the recombination fraction.

-
- B1 (Moo.) Dr. Graham, U. of N. Carolina. Mild. Note propositus a curiosity, unaffected but hypochondriacal.
 - B2 (Boo.) Dr. Graham, U. of N. Carolina. Severe.
 - B3 (Pru.) Dr. Graham, U. of N. Carolina. Moderate.
 - B4 (Har.) Dr. Graham, U. of N. Carolina. Severe-moderate.
 - B5 (Wil.) Dr. Graham, U. of N. Carolina. Mild. Part of S Kindred (Whittaker *et al.*, 1962; see also Graham *et al.*, 1962). Methods: Whittaker *et al.*, 1962.
 - B6 (Col.) Dr. Ingram, St. Thomas' Hosp. Med. School, Dr. Hardisty, Hosp. for Sick Children and Dr. Flute, King's Coll. Hosp. Med. School. Moderate.
 - B7 (Man.) Dr. Ingram, St. Thomas' Hosp. Med. School and Dr. Hardisty, Hosp. for Sick Children. Severe. History of 'bleeders' in grandmother's family: the Xg groups of the grandparents can be deduced. Methods: Rodman *et al.*, 1957 (adapted). Hardisty and Macpherson, 1962 (adapted).
 - B8 (But.) Dr. Holman, Group Lab., Lewisham. Severe. Methods: Biggs and Macfarlane, 1962.
 - B9 (Hit.) Dr. McKusick, Johns Hopkins. Mild.
 - B10 (Dut.) Dr. McKusick, Johns Hopkins. Mild. Methods: Margolius *et al.*, 1961.
 - B11 (Gam.) Dr. O'Brien, Cent. Lab., Portsmouth. Severe. Methods: Biggs and Macfarlane, 1962.

TABLE 2. X_g AND HEMOPHILIA B: THE LOD SCORES OF THE FAMILIES IN FIG. 3

	.05	.1	.15	.2	.25	.3	.35	.4	.45	.499
B 1 1 n-rec:11 rec	-10.721	-7.434	-5.523	-4.174	-3.135	-2.296	-1.591	-.988	-.465	0
B 2 3 n-rec	.837	.756	.690	.612	.528	.438	.342	.237	.123	0
B 2 2 n-rec:1 rec	-.442	-.189	-.063	.010	.051	.070	.073	.061	.036	0
B 3 z_1 3:0, e_1 3:0	.718	.604	.495	.391	.292	.201	.121	.057	.015	0
B 3 z_1 1:1, e_1 1:1	-.825	-.528	-.358	-.243	-.150	-.099	-.034	-.024	-.005	0
B 4 2 n-rec:2 rec	-1.442	-.888	-.586	-.388	-.250	-.152	-.082	-.036	-.010	0
B 5 z_1 3:2, e_1 3:2	-1.455	-.899	-.595	-.396	-.256	-.155	-.084	-.036	-.009	0
B 6 5 n-rec:2 rec	-.605	-.123	.104	.224	.278	.286	.260	.201	.113	0
B 7 1 n-rec	.279	.255	.230	.204	.176	.146	.114	.079	.041	0
B 8 z_1 3:1, e_1 3:1	-.483	-.241	-.126	-.064	-.030	-.012	-.003	-.001	-.000	0
B 9 z_1 7:5	-3.349	-2.004	-1.249	-.836	-.528	-.314	-.167	-.071	-.018	0
B 10 1 n-rec:1 rec	-.721	-.444	-.293	-.194	-.125	-.076	-.041	-.018	-.005	0
B 11 1 n-rec:1 rec	-.721	-.444	-.293	-.194	-.125	-.076	-.041	-.018	-.005	0
total lod score	-18.930	-11.579	-7.567	-5.048	-3.274	-2.039	-1.153	-.557	-.189	0
antilog = relative probabilities of θ						.009	.070	.277	.647	1.0
tentative <i>a priori</i> correction	.049	.050	.059	.078	.109	.156	.227	.338	.542	1.219
corrected sum	-18.881	-11.529	-7.508	-4.970	-3.165	-1.883	-.926	-.219	.353	1.219
antilog = relative probabilities of θ (corrected)						.013	.119	.604	2.254	16.560
family B 1 excluded										
total lod score, uncorrected	-8.209	-4.145	-2.044	-.874	-.139	.257	.438	.431	.276	0
antilog = relative probabilities of θ			.009	.134	.726	1.807	2.742	2.698	1.888	1.0

on the short arm of the X chromosome (Lindsten *et al.*, 1963; Stewart 1961) and, since the current estimate of θ for hemophilia A and deutan is only 0.12, hemophilia A would probably be on the short arm too. According to Lindsten *et al.* (1963) the argument for the Xg locus being on the short arm depends on the assumption that isochromosomes for the long arm are not genetically inert. These isochromosomes are known to be late-synthesizing (Muldal *et al.*, 1963; Gianelli, 1963) and since opinion is perhaps swinging towards the view that heteropycnotic X chromosomes are genetically inert, at any rate in mice (Ohno and Cattanaach, 1962), it seems safer, at present, not to apply a correction which would be appropriate to genes on the short arm. If the first three recombination fractions in table 3 are supported by further family tests (particularly $\theta = 0.26$ for Xg — G6PD) then the genes for Xg, G6PD, deutan and hemophilia A would, wherever they may be sited, span a length of chromosome not greater than that assumed for the short arm and an appropriate correction could then be applied.

About such corrections Dr. C. A. B. Smith contributes the following comment. "These corrections are obtained in the same way as the prior distribution of recombination fraction in Morton's (1955) paper. They represent an attempt to take into account our initial knowledge about the positions of the different loci on the chromosomes. Such initial information cannot be validly disregarded; for if we had good reason to believe that two loci are both on the short arm of the X chromosome, this would provide good reason for discounting, to an appropriate extent, any evidence that they are very loosely linked. If we only know that they are somewhere on the X chromosome, we will think differently. However, it is necessary not to over-estimate the importance of this correction, for various reasons. One is that different investigators may view the initial evidence rather differently, and hence may wish to use different corrections. Therefore in all cases the uncorrected anti-lod score, or 'likelihood value' is given: this

TABLE 3. ESTIMATES OF RECOMBINATION FRACTIONS
AND MAP DISTANCES

	recombination fraction, θ (with 90% confidence limits)				map distance in centimorgans		
	observed	corrected	limits	limits	Kosambi obs.	Kosambi corr.	Haldane corr.
¹ Xg:g6pd	0.26	(0.17-0.41)	0.26	(0.17-0.47)	29	29	37
² g6pd:deut.	0.05	(0.01-0.18)	0.05	(0.01-0.19)	5	5	5
³ deut.:hem.A	0.12	(0.06-0.26)	0.12	(0.06-0.27)	12	12	14
⁴ Xg:deut.	0.38	(0.24-0.5)	0.499	(0.27-0.5)	50	long	71
⁵ Xg:hem.A	0.40	(0.24-0.5)	0.499	(0.28-0.5)	55	long	80
⁶ Xg:hem.B	0.499	(0.37-0.5)	0.499	(0.42-0.5)	long	long	long
⁷ Xg:hem.fam.B1 excluded	0.37	(0.26-0.5)	0.499	(0.27-0.5)	47	long	67
(1)+(2)+(3)					46		56
(3)+(4)					57		78

¹Adam *et al.*, 1963: Israeli families: in which paper $\theta = 0.27$, but 3 maternal grandparents subsequently tested reduce θ to 0.26.

²Porter *et al.*, 1962, 1963: Negro families. See also Siniscalco *et al.*, 1960 and Adam, 1961.

³Calculations based on the pedigree of Whittaker *et al.*, 1962: Caucasian family.

⁴Calculations based on the pedigrees of Jackson *et al.*, 1963: Caucasian families.

⁵Present paper.

summarizes the evidence provided by the families in question, as distinct from external evidence incorporated into the prior distribution. In the second place, the *a priori* correction will only make any appreciable difference to the estimate if the information is small. This is in accordance with common sense, and means that different investigators starting with different assumptions will nevertheless come to practically the same final conclusion if there is adequate evidence provided by the sample; but with only a comparatively small sample they may not be brought into close agreement."

Dr. J. H. Edwards further points out that "the *a priori* corrections used assume that the number of chiasmata observed on the autosomes of men (about sixty) can be used to estimate the recombination fraction of the X chromosome in the ovary during fetal life, that the arbitrary level of interference postulated by Kosambi is appropriate, and that the probability of recombination cannot exceed one-half.

"In practice the corrections do not make much difference if the recombination fraction is less than one-third; if it is more than one-third then its exact value is of little consequence since the estimate is likely to be so vague, compared to estimates related to intervening loci, that the information contributed to mapping is relatively small."

MAP DISTANCES

In order to correlate recombination fractions it is necessary to convert them into map distances. To do this a correction has to be applied to allow primarily for double crossing-over, the products of which will be scored as non-recombinants. Two formulae are available:

(1) Kosambi (1944): $w = 0.5757 \log_{10} \left(\frac{1 + 2\theta}{1 - 2\theta} \right)$ where θ is the recombination fraction and w is the map distance in morgans.

(2) Haldane (1919); $w = -1.1514 \log_{10} (1 - 2\theta)$.

The available estimates of recombination fractions, observed and corrected, and the corresponding map distances are given in Table 3. The map distances, though diverging in the higher ranges of θ , are at least consistent with the order:

Xg — G6PD — deutan — hemophilia A

adumbrated by earlier work with anti-Xg^a (Race and Sanger, 1962). No doubt work which is continuing will modify all the distances but the order may be correct.

The recombination fractions between Xg and hemophilia A and B are too high for two-generation families to be able to give any further useful information. Families are now needed in which the maternal grandfather, or occasionally maternal grandmother, discloses the coupling or repulsion phase of the relevant genes. Even so the contribution of further data may be small. More useful information is likely to be obtained from data on the distances between deutan, protan and Xg, between deutan and hemophilia B, and protan and hemophilia A.

SUMMARY

An investigation of the linkage relations between the genes responsible for the X-linked blood group system Xg and for hemophilia A and B is recorded. Thirty five families with hemophilia A (factor VIII deficiency) and 20 families with hemophilia B (Christmas disease, factor IX deficiency) were tested with anti-Xg^a and the results were analysed by the lod score method: the estimate of the recombination fraction between Xg and hemophilia A is 0.40 (with wide confidence limits) and that between Xg and hemophilia B is 0.50; however, if one peculiar family is removed the estimate for Xg and hemophilia B becomes 0.37, again with wide confidence limits. The problem of a suitable correction to allow for the *a priori* odds against close linkage is discussed. Translated into map distances the uncorrected estimate of the recombination fraction between Xg and hemophilia A is compatible with the sum of the calculated distances between the supposedly intervening genes, and the map order Xg — G6PD — deutan — hemophilia A is supported.

ACKNOWLEDGMENTS

We thank Dr. J. H. Edwards, Dr. J. H. Renwick, Dr. Sheila Maynard-Smith and Dr. C. A. B. Smith for much patient coaching and guidance in the statistical treatment of the data. Dr. C. A. B. Smith also provided tables of z_1 and e_1 values for recombination fractions of 0.15, 0.25, 0.35 and 0.45. We thank Dr. P. T. Flute of King's College Hospital Medical School for sending some of the samples from family B6. We also thank Dr. R. D. Popham of Bury and Dr. E. K. Blackburn of Sheffield who sent samples of blood from families which are not included in this report because the Xg groups did not segregate informatively.

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The Absence of Close Linkage of Methemoglobinemia and Blood Group Loci

EDWARD M. SCOTT,¹ MARION LEWIS,² HIROKO KAITA,²
BRUCE CHOWN² AND ELOISE R. GIBLETT³

¹*The Arctic Health Research Center, U. S. Public Health Service, Anchorage, Alaska*

²*The Rh Laboratory, Winnipeg, Canada*

³*The King County Central Blood Bank, Seattle, Washington*

HEREDITARY METHEMOGLOBINEMIA due to a limited capacity of the red cell to reduce methemoglobin (Gibson, 1948) has been shown to be due to complete absence of a DPNH diaphorase in the cells (Scott and Griffith, 1959). It is possible by enzymatic means to distinguish with fair certainty heterozygous from normal individuals (Scott, 1960). An attempt was therefore made to test for possible genetic linkage between the enzymatic type of methemoglobinemia and several blood group loci.

METHODS

The population studied consisted of 49 families with evidence of the recessive gene for methemoglobinemia. With one exception (a Navajo family) all were Athabaskan Indians or Eskimos, with some Caucasian admixture. DPNH diaphorase was determined as previously described (Scott, 1960). Transferrin and haptoglobin types were determined by starch gel electrophoresis as described by Smithies (1959). Probability of linkage was calculated by the method of Morton (1955).

RESULTS AND DISCUSSION

In 33 families, at least one parent was heterozygous both for methemoglobinemia and for one or more blood group loci. All samples tested were Lu(a-b+), Mi(a-), Vw-, Be(a-), Wr(a-), and Di(a-), and all had type C transferrin. The results on the 33 families are summarized in Table 1. Except for the Kell locus, where insufficient data are available, linkage between methemoglobinemia and any of the loci must be quite loose if it occurs. It is possible that a linkage of Rh and methemoglobinemia with a recombination fraction of about 0.3 might be demonstrated if a larger number of families could be studied.

The difficulty in classifying a small fraction of the samples as heterozygous for methemoglobinemia or as normal has been previously discussed (Scott, 1960). In this study, no family was included unless evidence of the recessive

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TABLE 1. PROBABILITY OF LINKAGE OF ENZYMATIC
METHEMOGLOBINEMIA AND BLOOD GROUP LOCI

Locus	No. of Families	No. of Children	Log Probability of Linkage for Recombination Fraction of:				
			0.05	0.1	0.2	0.3	0.4
ABO	9	32	-5.3	-3.1	-1.2	-0.4	-0.1
MNS	26	95	-16.5	-8.6	-2.6	-0.5	0
Rh	12	47	-3.2	-0.8	0.6	0.7	0.2
Duffy	5	19	-1.3	-0.4	0.1	0.2	0.1
Kidd	13	48	-6.4	-3.1	-1.0	-0.2	0
P	4	11	-1.0	-0.4	0.1	0.1	0
Kell	1	4	0.8	0.7	0.5	0.3	0.1
Haptoglobin	4	9	-0.7	-0.2	0	0	0

All samples tested with anti-A, B, A+B, A₁; M, N, S, s; C, C^w, c, D, E, e, f (ce); Fy^a, Fy^b; Jk^a, Jk^b; P₁; K, k, Kp^a, Kp^b.

gene was found in at least two family members. Only one case was found of a child, apparently heterozygous for methemoglobinemia, with normal parents. This family and the child with the possible recombination of MN and S genes (Lewis *et al.*, 1963) were excluded from the study.

SUMMARY

Evidence was sought for genetic linkage of hereditary methemoglobinemia of the enzymatic type and the blood group loci ABO, MNS, Rh, Duffy, Kidd and P and the haptoglobins. From the study of 33 families, it is concluded that linkage with any of these loci is unlikely and must be quite loose if it occurs.

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LETTER TO THE EDITOR

BLOOD GROUP FREQUENCIES IN MONGOLS

In an effort to determine whether any of eight loci are on chromosome No. 21, we recently reported blood and saliva types of 793 mongols and 1,508 controls (Shaw and Gershowitz, *Amer. J. Hum. Genet.* 14: 317-344, 1962). A slight decrease in the frequency of group O mongols was found to be significant at the 5 per cent level when compared to the controls.

An additional 207 mongols (130 males and 77 females) residing in Mount Pleasant State Home and Training School, Mount Pleasant, Michigan, have now been tested. By coincidence, the total mongol sample is now increased to exactly 1,000.

The results of the blood and saliva tests are given in table 1. ABO comparisons with the previous sample and controls are given in table 2. It can be seen that, with the increased sample size, the deficiency of blood group O among the mongols is now below the 2 per cent level of significance. Furthermore, most of the chi-squares are larger than before, indicating that the ABO blood group distribution of the newly-tested trisomic population shifts from that of the diploid population in the same direction as noted previously.

These findings increase the probability that mongols do, in fact, differ in their ABO blood group distribution from that of the normal controls. Further-

TABLE 1. RESULTS OF BLOOD AND SALIVA TYPINGS OF
207 INSTITUTIONALIZED MONGOLS

Locus	Phenotype	No.	%	Locus	Phenotype	No.	%
ABO	A ₁	75	36.2	Rh	R ₁ r	71	
	A ₂	19	9.2		R ₁ w _r	1	
	B	22	10.6		R ₁ R ₁	38	
	A ₁ B	10	4.8		R ₁ R ₂	29	
	A ₂ B	1	0.5		R ₂ r	19	
	O	80	38.6		R ₂ R ₂	6	
					R ₀	3	
MNSs	MS	12	5.8		(total rr	40	19.3)
	MSs	26	12.6		rr	36	
	Ms	23	11.2		R'r	2	
	MNS	6	2.9		R ₁ ^u r	1	
	MNSs	43	20.9		R ₀ ^u	1	
	MNs	47	22.8	KELL	KK	0	0
	NS	0	0		Kk	17	8.3
	NSs	12	5.8		kk	189	91.7
	Ns	37	18.0		NT	1	
	NT	1		KIDD	Jk ^b (+)	10	
P	P(+)	160	77.3		Jk ^b (-)	6	
	P(-)	47	22.7		NT	191	
DUFFY	Fy ^a (+)	138	67.3	SECRETOR	ABH		
	Fy ^a (-)	67	32.7		secretor	147	71.0
	NT	2			non secretor	60	29.0

All bloods were Mg, Wr^a, and V^w—negative. All P-negative bloods were Tj^a-positive.
NT — not tested.

TABLE 2. COMPARISONS OF ABO BLOOD GROUPS OF 1,000
INSTITUTIONALIZED CAUCASIAN MONGOLS AND 1,508 CONTROLS

Population	A	B	AB	O	Total
Mount Pleasant mongols	94 (45.4)	22 (10.6)	11 (5.3)	80 (38.6)	207
Previous report	349 (44.0)	94 (11.9)	25 (3.2)	325 (41.0)	793
Total mongols	443 (44.3)	116 (11.6)	36 (3.6)	405 (40.5)	1000
Controls	621 (41.2)	144 (9.6)	55 (3.6)	688 (45.6)	1508
Comparison	χ^2	D.F.	P	χ^2 (Previous report)	
O vs. non O	6.254	1	.01 - .02	4.540	
O vs. A	5.518	1	.01 - .02	3.353	
A vs. non A	2.396	1	.10 - .20	1.707	
B vs. non B	2.722	1	.05 - .10	2.977	
AB vs. non AB	0.003	1	.95 - .98	0.379	
A vs. B vs. AB vs. O	7.449	3	.05 - .10	6.563	

Figures in parentheses are percentages.

more, we feel that the hypothesis that the ABO locus is on chromosome No. 21 has not been excluded. However, other explanations (such as differences in parental ABO frequencies, "disease association," and chance) must also be entertained.

Since large numbers of institutionalized mongols are easily accessible, we hope that these findings will stimulate others to determine the blood group frequencies of mongols in other geographical regions, and compare them with suitable controls. In addition, the testing of parents and sibs of mongols deserves strong consideration in determining the significance of these data.

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MARGERY W. SHAW AND HENRY GERSHOWITZ
Department of Human Genetics,
University of Michigan Medical School,
Ann Arbor, Michigan

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